CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY
OF THE MUSEUM OF COMPARATIVE ZOÖLOGY
AT HARVARD COLLEGE.— No. 240.

STUDIES ON THE PERIPHERAL NERVOUS SYSTEM OF AMPHIOXUS.

By Harriet Lehmann Kutchin.

Presented by E. L. Mark. Received June 2, 1913.

Table of Contents.

	Page
Introduction	571
Literature	572
Material and Methods	572
Dorsal nerves	574
A. Nerves of the rostrum	574
B. Nerves of the buccal region	579
C. Nerves of the velum	589
D. Nerves of the branchial region	593
E. Nerves posterior to the atriopore	603
F. Spinal ganglia	606
G. Structure of the dorsal nerves	607
H. Sensory endings of dorsal nerves	608
Ventral nerves	615
Bibliography	621
Explanation of figures	626

Introduction.

The literature on the nervous system of Amphioxus presents such divergence of opinion that it is probable many facts with regard to the structure, distribution and functions of this system still remain to be determined and illustrated. It will be impossible to more than theorize as to the primitiveness or the degeneracy of the nervous system of Amphioxus until the main features of its organization are more clearly understood. The present research aims to contribute toward a knowledge of the structure and distribution of the peripheral nerves, and it is hoped may aid in furnishing a basis for comparison with other vertebrates.

The following studies were undertaken in 1904 by the writer while holding the Alice Freeman Palmer Fellowship of Wellesley College.

Contributions from the Bermuda Biological Station for Research. No. 28.

I wish to express my sincere thanks to that institution for the exceptional opportunities afforded by the fellowship. Professor E. L. Mark of Harvard University suggested this field for investigation, and I am also indebted to him for kindly advice and criticism. The work was carried on in 1904–1905 at the Bermuda Biological Station and in the Harvard Zoölogical Laboratories while I was registered as a Graduate Student in Radcliffe College. In the spring of 1905 the problem was continued at the Naples Zoölogical Station, through the kindness of the "Association for Maintaining the American Women's Table at the Zoölogical Station at Naples and for Promoting Scientific Research by Women." I am under obligation to those in charge of the Naples Station for many courtesies extended throughout my stay. During the year 1905–1906 laboratory privileges were kindly afforded me for further work in the Zoölogical Laboratories of the University of Chicago.

Two species have been used as a basis for study, the Caribbean Amphioxus, Branchiostoma caribaeum, found in Bermuda waters, and the form so abundant at Naples, Branchiostoma lanceolatum.

Literature.

Retzius (**'91**) and Dogiel (**: 02**) have reviewed the literature on the peripheral nervous system of Amphioxus in considerable detail, presenting particularly points where lack of agrreement exists. Other authors, as Rohde (**'88**) and Heymans et van der Stricht (**'98**), give historical accounts in connection with their personal observations. It is therefore unnecessary to give here a connected discussion of the literature; the work of the several authors will be taken up in connection with each question in its bearing upon the descriptive part of this paper. An extensive bibliography of Leptocardii is given by Lönnberg (**: 01 —**, pp. 206–214).

Material and Methods.

At the Bermuda Biological Station the tissues of a large number of Branchiostoma caribaeum were impregnated with methylene blue by the *intra-vitam* method. Gold chloride and the methods of Golgi were also extensively used. Many variations were employed in the use of all these methods, with the view of bringing out details of structure,

and these methods were found to give excellent results. Material was also hardened in various fixing fluids, 10% formol proving to be particularly valuable. The same methods were employed at Naples upon Branchiostoma lanceolatum, but a larger number of preparations was made, to provide against the uncertainty of impregnation. Further discussion of these methods will be taken up in the body of this paper, but a few general remarks are in place here.

The best impregnations of both superficial and visceral nerves were obtained by immersion of the living animals in sea-water colored a moderately dark blue with a stock mixture of ½% to 1% of methylene blue in normal salt solution. The sea-water mixture should never be opaque. It is probable that the small quantity of salt in the mixture causes the epithelium to loosen, thus permitting more direct action of the methylene blue. Such preparations are not, of course, suitable for study of sensory endings in the skin. Specimens immersed in this mixture do not appear impaired in vigor at the end of two or three hours. The subsequent exposure to air recommended by Dogiel (: 02) is of great importance; the length of time required for different nerves can be determined only by continued observation under the microscope. The specimen must be kept moist with the methylene-blue mixture during such exposure. Fixation was usually effected by the ammonium-picrate method, and it was found that the addition of a few drops of 1% osmic acid to each 100 cc. of the ammonium picrate used for fixing these preparations greatly aided in their preservation. Material treated in this way, preserved in the usual ammonium-picrate and glycerine mixture, and carefully guarded against unnecessary exposure to light, was found in excellent condition at the end of two years, while other preparations fixed without osmic acid were practically useless after one year.

The gold-chloride method recommended by Hardesty (: 02) for use after fixation with 10% formol proved useful in demonstrating motor fibers and their endings. I regard this method as worthy of wider use because of its accurate fixation and the comparative absence of artifacts. The action of the gold chloride is, however, no more certain in this method than in others.

Mallory's (: 00) method for study of the central nervous system proved useful in the case of Amphioxus for the peripheral nervous system. Vom Rath's fluid (strong) was employed, but with indifferent results, except for the central nervous system. The methods of Golgi for impregnation with silver, and their various modifications, are all useful in obtaining impregnations of different parts of the nerv-

ous system. The rapid method is usually most certain and reliable. Picro-carmine employed after treatment with very dilute osmic acid is suitable for only the thinnest tissues. Structures much beneath the external surface receive absolutely no fixation, and are therefore quite unreliable for study.

A Nernst electric lamp, or carefully adjusted direct sunlight, was found to bring into view nerves which it was quite impossible to observe with the ordinary adjustments of light. This is particularly true of methylene-blue preparations of the whole animal, which show plexuses between dorsal nerves, and of thick Golgi preparations. With such strong light the nerves in thick parts of the body may be seen *in situ* with surprising clearness. This method has the disadvantage of soon tiring the eyes. Various methods of dissection were employed on impregnated and stained material for the study of the visceral nerves.

Dorsal Nerves.

A. Nerves of the Rostrum.

Nerve I.— The usually accepted first-nerve pair was first noted by Goodsir (**'41**), and has been repeatedly described. Owsjannikow (**'68**) interpreted it as the *trigeminus*. Schneider (**'79**) says that from its position the first nerve should be the *opticus*. Rohon (**'82**, p. 60) regards the first two pairs of nerves as analogous to the sensory elements of the *trigeminus*, the third pair to the corresponding (gleichnamigen) *facialis* of higher vertebrates. Later authors hesitate to compare the first nerve with any nerve of the higher vertebrates until some facts with regard to its function are established. Dogiel (**: 02**) designates the first nerve as purely sensory, since its branches ramify exclusively in the skin. The latter author has described in detail the manner of branching of this nerve in Branchiostoma lanceolatum, and defines the territory which it innervates as the end of the rostrum, with an occasional extension into the adjoining dorsal and ventral skin regions. Dogiel finds some variation in the size and manner of branching of the first nerve.

Edinger (**: 06**) describes a pair of nerves arising, like an olfactory nerve, from the base of the brain anterior to the nerve pair which is commonly designated as the first. My observations have not brought to light such a nerve, but I do not deny its existence, since I have not

used the silver-impregnation method of Bielschowsky, by means of which Edinger demonstrated the nerve fibers which he describes.

My observations with regard to the commonly accepted nerve I agree in the main with those of Dogiel. This nerve and its branches, as well as nerve II, exhibit peculiarities during the process of *intra-vitam* impregnation with methylene blue. Nerves I and II usually become colored in the course of 20 to 30 minutes, before the sensory nerves of the thicker parts of the body do. If the immersion in methylene blue is continued, these anterior nerves shortly lose their color, but if examined after a considerably longer immersion ($1\frac{1}{2}$ to 3 hours), nerves I and II again appear impregnated, frequently with great brilliancy. This indicates that there is more than one epoch in the course of immersion in methylene blue when a successful impregnation of the anterior sensory nerves may be obtained. Another possibly significant fact with regard to the physiological character of the first two sensory nerves is that the methods of Golgi repeatedly produced no impregnation in these nerves in specimens whose sensory nerves were otherwise well impregnated.

Figures 1 to 5 are drawn to show the distribution of nerve I in Branchiostoma caribaeum. These furnish a basis for comparison with the previously published figures of Branchiostoma lanceolatum. Figure 6 (Pl. **2**) shows the exit of nerve I from the neural tube in B. caribaeum, and Figure 11 (Pl. **3**) presents its exit in B. lanceolatum. A comparison of the two species shows no striking differences in the manner of branching of this nerve, nor in the territory which it innervates. I find, as does Dogiel, that some variations occur in the size of this nerve, and in its area of distribution. In the same specimens complementary variations are to be noted in the manner of distribution of the branches of nerve II.

In Figures 11 and 12 particular care has been taken to illustrate the relation of the first two nerves to the most anterior myomere. These figures represent the rostrum without its epithelial covering, this having sloughed off in the course of treatment following immersion in methylene blue. The fact that the main trunk of the first nerve usually gives off no branches for a considerable distance after its exit from the neural tube is of interest as distinguishing it from all other dorsal nerves. The cells of Quatrefages have no definite arrangement with relation to the nerves of the rostrum, as may be readily noted by comparison of Figures 1 to 5.

The main stem of nerve I, as well as the anterior end of the nerve cord, does not bear a constant relation to the chorda dorsalis nor to

the anterior portion of the first myomere. Occasionally little of the main stem of nerve I is visible; in other cases it may be observed for almost its entire length, while in still other specimens its union with the neural tube, and even a portion of the tube itself, are exposed to view. The main stem usually lies close to the dorsal surface of the chorda.

Nerve II.— In 1841 Johannes Müller described the second nerve, enumerating it as the first (J. Müller '**44**, p. 95, 96). Quatrefages ('**45**) numbered the branches of nerve II as nerves II, III, IV, and V, and figured the terminal branches of nerve I in the rostrum as arising from nerve II. This conception was based upon his idea that the first was an *optic* nerve, a short stalk connecting brain and "eye spot." Schneider ('**79**) figures (Taf. 15, Fig. 1) two roots for nerve II of the right side, stating (p. 14) that this nerve usually possesses two roots upon one side, and upon the other divides immediately after its origin.

Dogiel (**: 02**) gives an extended and accurate account of the distribution of the branches of nerve II, as well as the variations which occur in the form of the nerve itself, and the territory which it innervates. He finds from one to three nerves in the region of the exit of nerve II, conditions not being symmetrical in this respect on the two sides of the body. He enumerates these irregularly occurring nerves as distinct nerves, without considering their relation to the myomeres. Although his figures do not present the muscle segments with clearness, they convey an inaccurate idea of the number and position of these segments. Dogiel describes several forms of variation from the usual course to the rostrum of nerves I, II, and III (as he enumerates them). In one case nerve II is poorly developed, and a branch of his nerve III innervates the ventral part of the rostrum (Dogiel's Fig. 6*b*, which illustrates this condition, shows the roots of nerves II and III in close proximity). In another case, of frequent occurrence, nerve II is strongly developed and only the posterior ventral part, if any, of the rostrum receives branches of nerve III. (In Dogiel's Fig. 6*c*, and 6*d*, illustrating this case, the nerve designated as III is the one commonly accepted as III.) Occasionally his nerve III (shown in his Fig. 7, which is not the nerve usually enumerated as III) ramifies exclusively in the skin of the dorsal fin, a ventral branch being absent. In such cases the skin of the posterior ventral portion of the rostrum is supplied by branches from his nerve IV. (From Dogiel's Fig. 7 this is clearly the commonly accepted nerve III.) In another form of variation (Dogiel's Fig. 8*a*, and 8*b*) his nerve III consists of two or three dorsal branches and a small ventral ramus, which does not reach

the ventral border of the rostrum. According to his figures this nerve (III) makes its exit near the root of nerve II. In a few cases (illustrated by his Fig. 9) Dogiel observed directly posterior to the large root of nerve II two small nerves, which he designated as nerves III and IV. Neither of these possesses a ventral ramus which reaches to the ventral border of the rostrum; in such cases the skin of the rostrum is in part supplied by his nerve V. It is clear from his figure that the nerve numbered V is the one usually designated as III.

From the foregoing description it will be seen that Dogiel does not regard the anterior sensory nerves as occupying definite relations to the myomeres. Changes in the numbering of the anterior sensory nerves must of course affect the numbering of all the other sensory nerves. Thus one, two or three roots in the region of the exit of nerve II, if designated in accordance with Dogiel's view as separate nerves, would correspondingly change the numbering of all the succeeding dorsal nerves, which show such a constant relation to the muscle segments. Since the two sides are frequently not symmetrical with regard to the number of roots in the region of nerve II, one might have, according to Dogiel's system of enumeration, the correspondingly numbered nerves of the two sides belonging to myomeres *not* correspondingly numbered.

I have examined a large number of specimens, frequently with the aid of artificial light, and find that roots accessory to the main root of nerve II are often present, as is shown in my Figures 11 and 12 (Pl. **3**). These two figures present the two sides of the same individual and illustrate the manner of exit of the roots of nerves II and III, and their relation to the myomeres. They also show the lack of symmetry in the roots of nerve II on the two sides of the body. Figures 6 and 7 (Pl. **2**) show other conditions in the roots of nerve II. These do not differ in their form and position from the nerves described by Dogiel for this region. The main root of nerve II, and those roots that make their exit in close proximity to it, always lie anterior to the first myomere of the adult. Small roots may lie considerably posterior to the main root of nerve II and still occupy a position anterior to the first myomere. This is possible owing to the form of the muscle segment. Examination of a large number of individuals leaves in my mind no doubt of this condition. A distinct myomere intervenes between the root (or roots) of nerve II and that of the commonly accepted nerve III (Figs. 11 and 12). Moreover, the territories innervated by these nerves, while subject to some variation, are comparatively well defined. The dorsal nerves make their exit in definite relation to the

myomeres, as is illustrated in Figure 6, and a logical enumeration of the nerves could not allow the association of the same myomere with different nerves in different individuals. Nerve III has a definite character and territory of distribution; its number should not depend upon the number of roots possessed by nerve II, nor on their being variously designated as nerves III, IV or V. It is not an unknown condition for a dorsal nerve in the more posterior parts of the body to have two distinct roots making their exit through the same myoseptum. The possible cranial character of the nerves anterior to the first myomere in the adult can scarcely authorize the enumeration of each separate root in the region of the place of exit of nerve II as a distinct nerve root; because the lack of symmetry on the two sides of the body, and the irregularity of the occurrence of these roots, would leave no basis for rational enumeration. The fact is that nerve II supplies a fairly definite territory, consisting usually of a large part of the rostrum and an adjoining portion of the dorsal fin.

I have not observed the small muscle segments which Dogiel figures (his Fig. 6*a*, 6*b*, 8*b*) anterior to the origin of nerve II. The anterior boundary of the first muscle segment of the adult is often difficult to distinguish, and in my own observations strong artificial light was frequently employed to make this clear. Careful camera drawings were made of the dorsal portions of the anterior myomeres, as shown in Figures 6, 11 and 12. In specimens of both species the anterior projection of each muscle segment forms a much more acute angle than Dogiel's figures indicate. In this connection Hatschek's (**'92**) views regarding the first myomere may be mentioned. He states that in the larvae of Amphioxus the muscle fibrillae of the rostral process are well formed even out to the tip of the rostrum; but in the fully developed animal they become rudimentary, only a remnant persisting. In transverse sections of young Amphioxus (not larvae) treated with Mallory's differential stain, I have observed the cut ends of muscle fibers, lying in front of the anterior border of the first adult myomere. These fibers were short and few in number, but clearly differentiated by the stain from the surrounding connective tissue.

Nerve II was studied to a considerable extent in Branchiostoma caribaeum, and its distribution in the rostrum of this species is shown in Figures 1–5. As this species has been little illustrated, it is thought best to reproduce the conditions rather fully, for such figures form an interesting basis of comparison with Branchiostoma lanceolatum. No very essential differences, however, occur between the two species. Variations in the number and arrangement of the roots of nerve II

are probably as frequent in one form as in the other. Hatschek ('92, Fig. 6) figures a branch of nerve II as passing to the deeper nerve plexus of the mouth. I have never observed such a branch, but am aware that variations occur. However, such a condition can scarcely be the typical one. Nerves I and II usually send no branches to deep lying structures.

No new observations concerning the olfactory pit were made in the present study, and this structure has not been included in the enumeration of the dorsal nerves.

With regard to the spinal or cerebral character of the more anterior dorsal nerves, and especially those having no ventral roots, there has been much variation of opinion. Owsjannikow ('68) and Stieda ('73, p. 48) designate the first two nerve pairs as cranial. Stieda, however, denies the existence of nerves of special sense. Langerhans ('76, p. 279) says that the first and second nerves are distinguished from the others by the possession of peripheral ganglion cells, as well as by their origin. Schneider ('79, p. 14) regards the first and second nerves, and the bulbus olfactorius as cranial, but considers it uncertain whether they differ in function from the remaining sensory nerves. Rohde ('88) calls the first five sensory nerve pairs cerebral. It does not fall within a discussion of the distribution and structure of the peripheral nerves, to treat of the question as to the cranial or spinal character of the more anterior dorsal nerves. The structure of the neural tube, and the functions of these nerves must enter largely into such a determination. If, as Hesse ('98) states, the whole spinal cord reacts to light stimulation, we may well make careful investigation of the physiological character of the more anterior nerves, before ascribing any special degree of cephalization to Amphioxus. Heymans et van der Stricht ('98, p. 68) say, in regard to this question, that the anterior extremity of the spinal cord of Amphioxus might perhaps be considered anatomically as a head (in its first stage of development), but that physiologically the head perhaps extends over the whole nerve axis, though especially developed in the segment corresponding to the buccal and branchial region.

B. Nerves of the Buccal Region.

Nerve III.— The third dorsal nerve usually sends a small branch, or branches, to the posterior part of the rostrum, but by far the greater part of its branches are confined to the buccal region. It may, therefore, be properly included with the nerves of this group.

Certain earlier authors, as Schneider ('79) and Rohon ('82), regarded the third pair of dorsal nerves as the first true sensory spinal nerves, this interpretation being based partly on the fact that the first pair of motor roots is associated with this pair of sensory roots. These authors enumerated the "spinal nerves" separately, designating the third sensory pair as the first spinal sensory roots. At present it seems safest to enumerate this pair of dorsal nerves as the third, without attempting to distinguish between so-called cranial and spinal nerves.

Heymans et van der Stricht ('98) have given a careful account of the distribution of the branches of this pair of nerves in Branchiostoma lanceolatum, and Dogiel (: 02) discusses nerve "III" in the same species at considerable length. As has been noted, however, Dogiel ascribes no definite territory of distribution to the third pair of nerves, its number depending upon the number of roots present near the place of exit of nerve III from the neural tube. This uncertainty makes comparison difficult and much involved. It may be stated, however, that in his discussion of the innervation of the border of the mouth Dogiel designates the commonly accepted third nerve as III, as is shown in his Figur 1.

I have studied this nerve in both species, chiefly upon specimens impregnated with methylene blue, although gold chloride and picrocarmine bring out excellently many details in the nerves of the oral hood. The finer branches of the nerves of the buccal region are usually impregnated in from 15 to 30 minutes in the methylene-blue mixture previously described, while the main trunks of these nerves require a longer immersion. A rather long subsequent exposure to air (20 to 30 minutes) gave peculiarly brilliant results for the finer branches. Fixation with ammonium molybdate and osmic acid, after Dogiel's (: 02) method, is well adapted to this region. The thin tissues fix rapidly, and may be dehydrated quickly without loss of impregnation. It is advisable before fixation to detach the anterior portion of the animal, cutting a little behind the velum. By dissecting along the mid-dorsal line, a flat, fairly thin preparation may be obtained for mounting in balsam. Thick pieces of tissue fixed in ammonium molybdate are usually unsatisfactory for sections or other methods of study.

In the two species studied surprisingly little difference was found in the distribution of the branches of the third pair of nerves. Variations occur as frequently in Branchiostoma caribaeum as in B. lanceolatum, and are similar in character. In both species nerve III usually

possesses a dorsal and a ventral ramus, although in rare cases either may be absent, with the possible exception of the ventral ramus of the left side. I was unable to find any specimens in which the latter branch was entirely lacking. Occasionally the root subdivides at or near its place of exit from the neural tube, and either ramus is represented by two or more branches. The primary division in nerve III usually occurs near the neural tube, but considerable variation exists in this regard. Different forms of division in the two species are illustrated in Figures 6, 7, 11 and 12. In these figures the comparative uniformity in the place of exit of the nerve through the myoseptum may be noted.

The curious post embryonic history of the buccal region brings about the well known asymmetry in certain of the buccal nerve pairs. The dorsal ramus of the third nerve of either side usually innervates the neighboring portion of the dorsal fin, and a small area overlying the adjoining trunk muscles. A small branch of the ventral ramus of nerve III on either side of the body supplies the posterior ventral portion of the rostrum. Twigs from this branch may anastomose with branchlets of nerve II. In its course over the side muscles, this more anterior ventral branch of nerve III gives off numerous subdividing side branches. The main branch of the ventral ramus of either side takes a course ventrad and noticeably anteriad over the side muscles, and also gives off a number of side branches destined for the skin of this region. In this part of its course, or at the ventral border of the trunk muscles, this main branch of the ventral ramus of the third nerve, on either side of the body, gives off a branch (or branches) which passes to the anterior portion of the border of the mouth of the corresponding side and breaks up into the outer mouth plexus of this region. This branch usually leaves the more ventral side of the main branch, but may vary considerably in size and position. The main ventral branch of left nerve III is usually of greater size than the corresponding branch of the right side, and supplies a larger territory. This main branch divides at or near the ventral border of the trunk muscles of the left side into two branches, one of which passes beneath the ventral border of these muscles and emerges on the right side of the body, where it is continued across the oral hood to form the anterior portion of the inner mouth plexus of this side. The other branch traverses the oral hood on the left side, and breaks up into the left anterior portion of the inner mouth plexus. Thus the most anterior portion of this inner plexus on either side of the hood, is supplied by branches from left nerve III, while the outer mouth

plexus of the same region is formed symmetrically from branches of nerve III, one the right nerve, the other the left. Heymans et van der Stricht ('98) also note this condition in Branchiostoma lanceolatum. Dogiel (: 02) describes in detail the structure of the plexuses of the mouth border. These plexuses will be briefly considered later in this paper. I found no branches of left nerve II joining the inner mouth plexus, as described by Hatschek; on the contrary, the specimens observed indicate that the second nerve pair is not concerned in the innervation of the deep-lying structures of the mouth border.

Interesting variations occur in the distribution of the branches of nerve III in both species. In one case (B. lanceolatum) a branch of right nerve III anastomosed with the branch of left nerve III on the right side, thus actually joining the inner mouth plexus. Ordinarily the ramifications of left nerve III in the inner mouth plexus on either side join each other near the median line, but in one specimen (B. caribaeum) no anastomosis occurred, thus presenting the curious condition of an incomplete "nerve ring" in the inner mouth plexus.

Nerve IV.— This nerve usually impregnates with methylene blue coincidentally with nerve III, but the portion extending over the trunk muscles often shows a better impregnation after longer immersion. Bilateral asymmetry exists in the fourth nerve pair, as in the third pair, and many variations occur in its manner of division, and the distribution of its branches. As shown in Plate 2, Figure 6 (B. caribaeum), nerve IV arches slightly more than nerve III immediately after its exit from the neural tube. Near its place of exit, this nerve divides into a dorsal and a ventral ramus. More than one dorsal ramus may be present, and the ventral ramus may subdivide near its origin; but I have never noted the entire absence of the ventral ramus, or its equivalent, on either side of the body. Occasionally the dorsal ramus of a sensory nerve is absent, or represented by a twig of practically no importance. In such cases its territory is supplied by branches of an unusually well developed dorsal ramus of one of the two adjoining nerves. The place of division of nerve IV into a dorsal and a ventral ramus may be readily noted in Figure 6. This place of division is often concealed by the muscles, but frequently may be ascertained by the aid of strong light, especially in methylene-blue preparations preserved in glycerine and ammonium picrate. Such preparations often become very transparent. Heymans et van der Stricht ('98) say that the bifurcation of the dorsal roots into dorsal and ventral rami is always made before exit from the myoseptum, except in the case of the last caudal root. According to my observa-

tions, the place of division in the first 6–8 anterior nerves may occur at, or slightly after, the exit of the nerve from the myoseptum, although it frequently occurs nearer the neural tube.

The stem of the dorsal ramus of nerve IV is usually deflected more or less anteriorly (Figs. 6 and 7, Pl. **2**). This ramus divides repeatedly, distributing its branchlets over the surface of the neighboring trunk muscles, and in that portion of the dorsal fin lying approximately dorsal to the nerve root. The ventral ramus of nerve IV is usually of greater size than the corresponding ramus of nerve III, especially upon the left side. This ramus of nerve IV takes a general ventrad course over the trunk muscles; but in its course over these muscles it forms an arch the convexity of which is directed anteriorly. This arch is frequently more prominent on the left side, and a like one may also be noted in the ventral ramus of nerve V (Pl. **2**, Fig. 7). Dogiel (: **02**) states that an arch is also formed in the course of the ventral ramus of nerve III, and occasionally in nerve VI. I, however, have observed a *marked* arch only in the course of nerves IV and V. On the left side this arch is accentuated by the fact that the branch of this ramus which innervates internal substructures bends toward the interior of the body. The ventral ramus of nerve IV gives off numerous subdividing branches in its course over the side muscles. On the right side it divides into two or more descending branches, which innervate a portion of the outer wall of the oral hood, and finally break up into a part of the outer mouth plexus. These descending branches may be formed either in the course of the ventral ramus over the trunk muscles, or at the ventral border of these muscles, or even in the oral hood. The branches which cross the oral hood usually give off only small side branchlets. The ventral ramus of left nerve IV innervates a much larger territory, and shows considerable variation in the number of its branches, and their manner of distribution. This ramus usually divides into its main branches near the ventral border of the side muscles, but often varies in this regard. A branch, or branches, to the outer plexus of the mouth may be given off from the main branch at any point in its course over the trunk muscles (Fig. 7). Other branches to this plexus may be given off at or near the place of main division mentioned above.

The remaining branches formed at this place of division are destined for less superficial structures. Of these, one or more take a course ventrad across the oral hood and form that part of the inner plexus of the mouth lying posterior to the portion formed by branches of left nerve III. The branches of left nerve IV which aid in the formation

of the inner mouth plexus lose their slender, thread-like appearance as they approach the plexus, and become flattened and band-like (Pl. **2**, Fig. 7, *i;* Pl. **4**, Fig. 18, *i.*). These branches lie interior to those (*o.*) forming the outer mouth plexus, the difference in focus increasing in their distal portions. The branches forming the inner plexus also lose their fibrous appearance in methylene-blue preparations as they approach the plexus, and appear granular; this change may be noted in all branches of dorsal nerves innervating deep-lying structures. At the place of main division of the ventral ramus of left nerve IV a very large branch is also given off, which bends at once beneath the ventral border of the trunk muscles, and passes to the right side of the oral hood. Here it may emerge at varying places, usually, however, near the ventral border of the trunk muscles of the right side, and often anterior to the ventral branches of right nerve IV (Fig. 18). This branch of left nerve IV subdivides in various ways in the right wall of the oral hood, and the branches thus formed break up into that portion of the inner mouth plexus of the right side which lies posterior to the part formed by the branch of left nerve III, previously described. No other nerve branches from nerves on either side of the body appear to be concerned in the formation of the inner plexus of the mouth border on the right side, in either species.

Another large, more posterior branch of left nerve IV leaves the ventral ramus at its main place of division. This branch bends beneath the trunk muscles toward the interior, and after reaching the median region ventral to the notochord, runs posteriad as a thick, straight trunk. In methylene-blue preparations in which epithelial structures are not retained, this nerve branch appears to end abruptly a short distance in front of the velum, and slightly to the right of the median line (Fig. 8, IV*a*). This nerve may correspond to the branch of nerve V described by Hatschek (**'92**, p. 144) as innervating the sensory groove of Hatschek. Much variation certainly occurs in the distribution of the branches of the dorsal nerves in the buccal region. In this connection it may be noted that Heymans et van der Stricht (**'98**, Pl. VI, Fig. 22) figure a branch of left nerve IV passing directly to the right side of the velum. I, however, have been unable to find any connection between this nerve branch and the nerves of the velum; but, in some instances another large branch of the ventral ramus of left nerve IV clearly did join in the innervation of the velum. This branch is usually given off from the ventral ramus of the latter nerve at or near the main place of division, and most frequently enters the velum on the left side of the body. In one specimen (B. lanceo-

latum), however, after taking a posteriorly directed course for some distance along the inner border of the trunk muscles of the left side, this branch turned dorsad running toward and a little beyond the median plane, where it entered the velum slightly to the right of this plane. This is the only instance observed of a left nerve branch entering the velum on the right side of the body. In some cases a branch of left nerve IV is *indirectly* connected with the innervation of the velum by anastomosis with a large branch of left nerve V which passes directly to the velum. These variations occur in both species, and make it difficult to define the territory innervated by the fourth nerve pair. Such variations no doubt account, in large measure, for the apparent disagreements between the various descriptions of this nerve.

Nerve V.— Nerve V (Pl. **2**, Fig. 7) is in many respects similar to nerve IV. It is usually of about the same size, and the ventral ramus forms an arch similar to that described for nerve IV. The dorsal ramus of nerve V innervates a territory corresponding to that of the dorsal ramus of nerve IV, but more posterior, and the portion of the ventral ramus taking a course over the trunk muscles gives off branches similar to those leaving this portion of the ventral ramus of nerve IV. The place of division of nerve V into a dorsal and a ventral ramus in B. caribaeum is illustrated in Figure 6. The fifth nerve pair is also bilaterally asymmetrical.

The ventral ramus of right nerve V usually divides at or near the ventral border of the trunk muscles into two or more branches, which cross the oral hood and form that part of the outer mouth plexus lying posterior to the portion formed from branches of right nerve IV. Occasionally only a single small branch of right nerve V joins the outer plexus of the mouth border. The ventral ramus of left nerve V is larger than that of right nerve V, and exhibits considerable variation in its manner of division. The main branches are usually given off at or near the ventral border of the lateral muscle. In Figure 7 (B. caribaeum) a branch to the inner mouth plexus may be observed leaving the ventral ramus about half way in its course over the trunk muscles. One or more branches usually pass to the outer mouth plexus from the main place of division of this ventral ramus, and a like number leave this place for the inner plexus of the mouth border. A band-like nerve branch is also given off from left nerve V near the ventral border of the side muscles, which frequently anastomoses with a branch of left nerve VI (Figure 8). This nerve branch is usually concerned in the innervation of the velum. Another form of con-

nection between nerves V and VI is illustrated in Figure 7. As has been stated, a branch of left nerve IV sometimes anastomoses with left nerve V in this region; left nerves VI and VII often show some form of connection with it in the neighborhood of the ventral border of the trunk muscles. Thus, with the slight connection sometimes occurring between nerves II and III, a basis is formed for the band-like nerve described by Hatschek ('92) as connecting nerves II–VII in this region. Hatschek's Figure 6 must be somewhat diagrammatic, however, since this condition is found only on the left side, and even here these connecting nerves are not constant in occurrence or size. In nearly all cases left nerve V sends one or more branches to the velum. These branches usually leave the ventral ramus near the ventral border of the side muscles (Figs. 7, 8), and enter the velum on the left side of the body. At least one of these branches forms an anastomosis with a branch or branches of left nerve VI, and frequently a similar anastomosis is formed with a branch or branches of left nerve VII. These anastomoses vary greatly in character; it is often difficult to determine the share of each nerve in the innervation of the velum. This is especially true when, as sometimes happens, branches are given off to the inner mouth plexus ventral to the anastomosis between branches of left nerves V and VI (Fig. 7).

Nerve VI.— This nerve requires only a brief description. A form of division of the trunk of this nerve in B. caribaeum may be noted in Figure 6 (Pl. 2). The dorsal rami of this nerve pair are similar to those of the fifth pair of nerves; their main branches are illustrated in Figures 6 and 7. The ventral ramus of the sixth nerve, both the right and the left, is usually smaller than the corresponding ramus of nerve V, but the cutaneous branches given off in its course over the trunk muscles correspond to those leaving the similar portion of nerve V. In both species the remaining branches of right nerve VI are distributed in the oral hood and external portion of the mouth border of the right side. The ventral ramus of right nerve VI divides near the ventral border of the trunk muscles into two or more slender branches, which take a general course ventrad across the oral hood, and break up into a portion of the outer mouth plexus. The corresponding ramus of left nerve VI sends a similar branch, or branches, to the outer plexus of the mouth border on the left side of the body. Left nerve VI frequently sends a branch, or branches, to the inner plexus on the left side of the mouth, but this is not uniformly the case. In several specimens examined branches of left nerves III, IV, and V formed the entire left portion of the inner mouth plexus. Heymans

et van der Stricht ('**98**, p. 36) state that the plexus of Fusari is formed from branches of left dorsal nerves III–VI, and perhaps VII. As has been stated, a branch of left nerve V usually anastomoses with a branch, or branches, of left nerve VI. From this anastomosis a branch passes to the velum, frequently uniting in its course with a branch of left nerve VII (Fig. 7). This branch to the velum may divide in various ways before entering the latter structure. Its branches, however, enter the velum on the left side of the body.

Nerve VII.— Figure 6 (Pl. **2**) shows this nerve leaving the neural tube slightly nearer its dorsal surface than nerve VI. The dorsal ramus of nerve VII on either side of the body is similar to that of nerve VI, except for a slight increase in size, due to the larger territory lying dorsal to the seventh nerve root. The ventral ramus of nerve VII on either side of the body does not differ from that of nerve VI in the region of the trunk muscles. Right nerve VII sends one or more branches to the posterior portion of the outer mouth plexus. These branches give off small side branches in their course across the oral hood. Nerve VII of the right side occupies a position considerably posterior to that of left nerve VII, and its ventral ramus frequently lies exterior to the velum. In such cases right nerve VII is usually the most posterior nerve of the right side sending branches to the outer plexus of the mouth border. In nearly all the specimens examined the branches of left nerve VII were found to be exclusively cutaneous in their distribution. This nerve sends branches to the outer mouth plexus of the left side, similar to the corresponding branches of right nerve VII. I have been unable to find a branch, or branches, of left nerve VII in either species directly connected with the inner plexus of the mouth border. As has been noted, Heymans et van der Stricht ('**98**) state that left nerve VII is perhaps concerned in the formation of the plexus of Fusari. This may be the case in rare instances, but is certainly not the usual condition. These authors also state that left nerve VII contributes toward the innervation of the velum. Dogiel (: **02**) also says that nerve VII not infrequently takes part in the innervation of the velum. The specimens examined by me revealed no large branches of left nerve VII passing directly to the velum. In a few cases a small branch, or branches, of this nerve anastomosed with branches of left nerve V or VI (Figure 7); therefore fibers from branch VII may take this course to the velum; but it is difficult to determine the course of nerve fibers after such an anastomosis.

Nerve VIII.— This nerve will be briefly considered here, although its branches, for the most part, supply the branchial region. It may

be noted in Figure 6 that this nerve leaves the neural tube very near its dorsal surface, and arches noticeably immediately after its exit. The dorsal ramus of nerve VIII on either side of the body, and the branches of this nerve pair in the region of the trunk muscles, are so similar to the corresponding branches of the two next anterior nerves, already described, that a separate description is unnecessary. I have been unable to find a branch of the ventral ramus of right nerve VIII which unquestionably joins the outer plexus of the mouth border. However, since branches of this nerve sometimes lie considerably anterior to the velum, it seems probable that they may occasionally contribute toward the formation of that plexus. Left nerve VIII frequently sends a small branch to this plexus (Fig. 7), but such a branch is as frequently lacking. None of the specimens examined showed a branch of left nerve VIII passing to the velum, as described by Dogiel (**: 02**).

The variation in the territory innervated by the individual anterior nerves is of considerable interest, since the branches of a nerve usually supplying a certain area, may be supplanted by those of another nerve which leaves the neural tube at some distance from the place of exit of the nerve ordinarily innervating the given territory. It appears from this that the same nerve does not always carry fibers to precisely the same area. The plexuses and communicating nerve bands of this region may have a bearing on this point, and lead to doubt as to which nerves actually supply certain territories. The relation of the nerve components of the neural tube to each other in this region must also be of interest. It is certainly a fact worthy of consideration that a great nerve, like nerve IV, leaving the neural tube at a definite place, may or may not take a prominent part in the innervation of the velum.

The plexuses of the mouth border have been frequently described, especially by Fusari (**'89**) and Dogiel (**: 02**), and will not be taken up in detail in this paper. Besides the inner and outer plexuses of this region, Dogiel describes a "Zwischengeflecht," lying between them and taking its origin chiefly from the outer plexus. The nerve branches taking part in the formation of the plexuses of the mouth border in the specimens of both species observed by me have been already enumerated. Figure 18 (Pl. **4**) illustrates a portion of the outer plexus in Branchiostoma caribaeum. This plexus does not appear to be essentially different from the corresponding plexus in B. lanceolatum. The inner plexus is also apparently similar in the two species.

C. Nerves of the Velum.

The nerves of this structure have been so little described and figured that they will be considered under a separate heading in this paper. Rathke, as early as 1841, mentions the velum, giving the number of tentacles as sixteen. Rolph (**'76**) notes that the velum carries about ten cirri. Rohon (**'82**), van Wijhe (**'93**), and Hatschek (**'92**) mention nerve branches passing to the velum; but the most detailed description of the innervation of this structure is found in the work of Heymans et van der Stricht (**'98**). These authors state that the voluminous circular nerve of the velum is formed from branches of left dorsal nerves IV, V, VI and VII, and appears to occupy about the middle part of the sphincter muscle, at the interior of which it gives off large branches. With regard to nerves passing to the velar tentacles, these authors state (p. 36): "nous avons également observé à l'intérieur des tentacules des fibres nerveuses que nous avons pu poursuivre jusque entre des cellules épithéliales." Contrary to van Wijhe, they found in their preparations no impregnation of sense cells in the velum. As mentioned in the discussion of nerve IV, Heymans et van der Stricht describe a posterior branch of left dorsal nerve IV which passes under the body of Hatschek, and reaches the right portion of the velum. These authors illustrate (Pl. VI, Fig. 22) the large nerve branches passing to the velum, and also its circular nerve. Dogiel (**: 02**) finds branches from left nerves IV, V, VI, VII, and even VIII and IX, taking part in the innervation of the velum. The branches of nerves IV and V are usually, however, most important. Dogiel's enumeration of the more anterior nerves must be considered in connection with this statement. This author shows (Taf. 20–21, Fig. 13) a portion of the plexus of the circular muscle of the velum. In this figure branches of left nerves V, VI and VIII form the velar plexus. In his Figur 1, branches from left nerves IV, V, VI and VIII pass to the velum. The branches of nerves VIII and IX which he finds occasionally passing to the velum are fine branchlets of the rami viscerali of these nerves.

My personal observations on the innervation of the velum were made largely from dissected specimens of Branchiostoma lanceolatum and B. caribaeum impregnated by the intra-vitam method with methylene blue. Most of these specimens were fixed in ammonium picrate, and preserved in a mixture of the ammonium picrate and glycerine. A few specimens were fixed in ammonium molybdate, dissected and mounted in balsam. In either case, a few drops of osmic acid added

to the fixing fluid appeared to aid greatly in preserving the impregnation. It was usually most convenient to use only the anterior portion of the body in studying the velum, severing the body a short distance behind that structure. This anterior portion was prepared for study under a dissecting microscope by cutting with fine scissors along the longitudinal axis, usually on the right side, as close as possible to the dorsal fin. Certain specimens were cut in different regions, so as to make it possible to determine the course of the nerve branches on both sides of the body. These preparations were arranged on a slide in the preserving mixture, with the aid of camel's hair brushes. The velar tentacles were arranged in the position showing the nerves to best advantage. Thin supports beneath the cover-glass were usually necessary to prevent injury. Strong light was found useful in following the deep-lying nerve branches, but the velar plexus, when impregnated, is usually visible with ordinary light. The velar nerves require a long immersion in the methylene-blue mixture ($1\frac{1}{2}$ to $2\frac{1}{2}$ hours), with a subsequent exposure to air of about one hour. Unless osmic acid is added to the fixing fluid, the epithelial covering of the velum sloughs off to a large extent.

Although considerable variation exists in the innervation of the velum, Figure 8 (Pl. **2**) presents a fairly typical picture of the branches of the dorsal nerves which pass to this structure, and the distribution of the nerves in the velum itself. Figure 9 shows the nerves of a large and a small velar tentacle in greater detail. These figures present the first published views of the entire plexus of the velar muscle, and of the nerves of the velar tentacles. These figures do not show the epithelial covering of the velum, except for occasional cells apparently remaining attached to nerve branches.

Figure 8 may be taken as a basis for the description. In this specimen branches of the ventral rami of left nerves V and VI supply the velum. A somewhat complicated alternation of anastomosis and division takes place between these branches as they pass to the velum. A large branch of left nerve V takes a course along the ventral muscle border to the ventral ramus of left nerve VI, and anastomoses with the latter. Two branches destined for the velum are given off near this place of union. The larger (α) takes a backward course along the ventral border of the trunk muscles for a short distance, and then bends around the muscle border to the inner surface of the side muscles, where it is continued posteriorly with considerable deflection dorsalward. As it bends toward the interior, this large branch gives off a much smaller one (γ), which passes posteriad and ventrad, dividing after

a short distance into two branches, which reach the velum a short distance ventral to the muscle border. These two latter branches unite in the velar muscle, and the band-like nerve thus formed may be followed in almost the entire circumference of the ring muscle, although its size varies considerably in different portions.

The smaller branch (β), leaving the place of anastomosis between the branches of nerves V and VI, takes a general course backward over the inner surface of the trunk muscles, running for a short distance dorsal to the larger branch. Near the velum these two nerve branches (α, β) unite, forming a short, thick trunk. This trunk divides into two short, diverging branches of equal size. The more dorsal branch (b') enters the velum about the width of two myomeres dorsal to the ventral muscle border of the left side, and is continued dorsad in the circular muscle of the velum as a slightly irregular, thick band. This band curves over to the right side of the velum, and breaks up into the velar plexus. The more ventral (δ) of these short, diverging branches continues ventrad in the ring muscle of the velum, losing its band-like character at the base of the fifth tentacle ventral to the border of the trunk muscles. These band-like nerves lie chiefly near the bases of the skeletal portions of the velar tentacles, but do not form a continuous "band nerve." The velar plexus lies, for the most part, between these thick, flat nerves and the free portions of the tentacles. A comparatively large branch passes to each tentacle obliquely from one of the band-like nerves; this oblique deflection is usually ventralward, but may vary. A single large tentacle in the mid-ventral region receives two main branches, one from the right side, and one from the left. The velar plexus is completed by numerous small branches, which anastomose to form an irregular, loose-meshed network between the band nerves of the circular muscle and their large branches which supply the tentacles. Toward the more external border of this plexus many apparently free-ending small nerve branches are visible. These may terminate in some form in the velar epithelium, or may possibly join a finer unimpregnated plexus.

The nerves of the velar plexus generally exhibit a flattened, granular appearance when impregnated with methylene blue, and their outlines do not present the clear-cut aspect noticeable in cutaneous branchlets of dorsal nerves. Their structure calls to mind that of the nerve branches forming the inner plexus of the mouth border, and the inner abdominal plexus. Apparent oval nuclei, or nucleated cells, may be frequently observed in the course of the finer branches of the velar plexus (Pl. **2**, Fig. 9).

The tentacles of the velum are usually alternately large and small, but this arrangement does not obtain with absolute regularity. Their number does not appear to be constant, but is in the neighborhood of fifteen. The innervation of the tentacles presents a beautiful appearance when impregnated with methylene blue. Figures 8 and 9 illustrate these nerves with different degrees of detail. From these figures it may be observed that each large nerve passing to a tentacle breaks up suddenly, at the base of its tentacle, into a great number of small branches, which divide, anastomose and interlace throughout the entire free portion of the tentacle. Such meshes as are formed are usually much elongated parallel to the long axis of the tentacle. These nerves of the velar tentacles are also granular in appearance, and occasional small nuclei or cells occur in the course of the nerve threads. Free nerve ends may be noted, but these must always be considered in connection with the fact that in methylene-blue preparations little epithelium favorable for studying the general innervation of the velar tentacles persists. In some instances small, darkly-staining thickenings of the nerve ends may be seen projecting beyond the border of the tentacles, or exterior to the network of nerves (Fig. 9, *c*). These may represent some form of nerve ending in the epithelium of the velar tentacles. The elaborate innervation of the tentacles suggests that many nerve endings may exist in them.

The variation mentioned in the nerves supplying the velum has no doubt led to the apparently conflicting statements with regard to the numbers of these nerves. In the specimens observed, only branches of nerves of the left side of the body passed to the velum, and these usually enter the velar muscle on the left side. A branch of left nerve IV has been described which entered the velum slightly to the right of a mid-dorsal line, but this case appeared to be exceptional. Nerve IV may send a branch directly to the velum, or a branch may anastomose with that of nerve V on its way to the velum. In Figure 7 branches of nerves VI and VII anastomose with the branch of nerve V passing to the velum. It is difficult to explain just what these anastomoses mean. Nerve IV sometimes sends a large branch to the velum, nerves V and VI usually send branches of considerable size, but nerve VII seldom contributes largely toward the innervation of the velum. The branch of left nerve IV indicated at IV*a* in Figure 8 appears to be associated with the nerves of the velum, but is actually entirely free from the velar muscle. I have frequently observed a branch of left nerve IV ending in this manner in this region. Variation no doubt occurs in this nerve branch, and it may therefore

correspond to the branch of nerve V described by Hatschek ('92, Figur 6, *N. S.*) as passing to the groove of Hatschek. Heymans et van der Stricht ('98) state that the N. recurrens of Hatschek ends at the velum, and has nothing in common with the branchial plexus. I have never observed a large branch from the buccal nerves reaching the branchial basket.

D. Nerves of the Branchial Region.

In this group are included the dorsal nerves lying between the velum and the region anterior to the atriopore. The term "branchial" is therefore unsatisfactory in some respects, but is perhaps the one most available for use in a description of the nerves. In B. lanceolatum this region is supplied by nerves VIII to XL, or XLI, inclusive, while in B. caribaeum it seems probable that nerves XXXVIII and XXXIX innervate the region of the atriopore. This indicates that the difference in the number of myotomes in the two species occurs, in part, in the branchial region. Dogiel (: 02) states that in B. lanceolatum nerve XLII is the most posterior nerve supplying the region of the atriopore. He describes the fine-meshed plexus ramifying about this opening, and finds that branches of nerves XXXVIII–XLII may take part in its formation. Variations certainly occur in these nerves, as in the case of other dorsal nerves.

The nerves of this region impregnate particularly well with methylene blue, but require a longer immersion than those of the anterior part of the body, especially in the case of the visceral branches. Strong light was very useful in following the finer nerve branches in the thicker portions of the body. Various branches of these dorsal nerves have been repeatedly described; the accounts, which are somewhat fragmentary, will be taken up in connection with the description of my personal observations.

The dorsal nerves of this region differ little from each other in their main characteristics, and in certain respects resemble those of the buccal region. It may be noted that, as seen in Plate 2, Figure 6, the arch formed by each dorsal nerve as it leaves the neural tube gradually increases in height toward the middle region of the body. Here the arch remains practically constant, but toward the posterior region it again diminishes in height, and at the extreme posterior end, as will be noted later, no arch is present. In the anterior portion of the branchial region the place of division of the dorsal nerves into dorsal and ventral rami is usually at, or just before, the exit of each

nerve from the myoseptum (Fig. 6). In the remainder of this region the place of division lies deeper, due, in the main, to the greater thickness of the trunk muscles; for the majority of the nerves of this group divide at a comparatively uniform distance from the neural tube. Much variation exists with regard to the place of exit of the dorsal and ventral rami through the myoseptum (Pl. **6**, Fig. 34; Pl. **7**, Figs. 37, 39). Johnston (: **05**) states that "the typical place of division is about half way between the cord and the dermis," rather than close beneath the dermis as described by Hatschek. Johnston finds much variation in the place of division, and frequent cases of separate emergence of the dorsal and ventral rami.

The dorsal rami of the nerves of this group resemble those of the more anterior region. They are largest in the thickest portion of the body, and possess a great number of branches, and, like the anterior dorsal rami, innervate the cutaneous area over the neighboring trunk muscles, and the adjoining portions of the dorsal fin. These dorsal rami frequently emerge from the myoseptum not as single stems, but in several branches, division having taken place beneath the dermis (Figs. 34, 39).

The ventral rami of these nerves are usually large, but may vary, one or two rami being increased in size at the expense of an adjoining ramus (Fig. 39). A ventral ramus may even be entirely absent. Both dorsal and ventral rami give off a number of repeatedly dividing branches in their course over the trunk muscles. In many methylene-blue preparations which retain little epithelium, the finer branches appear to end in innumerable delicate branchlets, the distal ends of which are often noticeably directed outward. Frequently these slender branchlets are continued into fine threads, which anastomose with one another and with similar threads proceeding from adjoining nerves, forming a delicate, irregularly meshed plexus. Such plexuses are not limited to this region, but are also present in connection with nerves posterior to the atriopore. These plexuses have been so little observed and figured that a detailed account will be given of their distribution and character in connection with the discussion of sensory nerve endings. The ventral rami of the dorsal nerves of the branchial region (with a few possible exceptions) divide near the ventral border of the side muscles, to form three main branches (see sketch, Pl. **4**, Fig. 15a). This division is accomplished in various ways, and each resulting branch may be represented by one or more twigs. The most exterior branches formed by this division are the *rami cutanei ventrales*, noted by early authors, and well described by Fusari (**'89**),

Heymans et van der Stricht (**'98**) and Dogiel (: **02**). These branches ramify over the metapleural fold and ventral wall of the atrium, forming a cutaneous network, the meshes of which are generally elongated longitudinally. Nerve VIII is usually the most anterior nerve sharing in the formation of this plexus. Fusari (**'89**) describes nerve cells and ganglionic knots in connection with this network. It is of interest to note that these cutaneous abdominal branches may leave the ventral ramus at or near the ventral muscle border, or the ventral ramus may divide much earlier in its course, sending one or more twigs from the branches thus formed to the superficial abdominal region (Figs. 37, 39). In Figure 39 the absence of a ramus cutaneus ventralis in connection with nerve XXXVI is to be noted.

The remaining two divisions of the ventral ramus are frequently formed from one main short stem; this may bend toward the interior beneath the ventral border of the trunk muscles, or may penetrate a myoseptum at some distance (the width of one, two or even three myomeres) dorsal to this ventral muscle border. These two branches may, however, leave the ventral ramus independently, instead of by a common stem. On the inner surface of the trunk muscles the short stem mentioned above divides into two main branches. One of these, the ramus visceralis ascendens, ascends over the inner surface of the trunk muscles, while the other, the ramus visceralis descendens, descends to the inner surface of the transverse muscles. Nerves VIII or IX, and the succeeding dorsal nerves of this region usually possess a ramus visceralis descendens, but I was unable to find a ramus visceralis ascendens anterior to that of nerve XII. The most anterior ascending visceral ramus is small, but the succeeding one is larger, showing the characteristic fan-like branching. It is entirely possible that branches pass to the most anterior branchial bars from the descending visceral ramus, or the inner abdominal plexus. In this connection it may be noted that the fan-like branches of the most anterior ascending visceral rami observed, were directed obliquely forward. The diagrammatic sketch, Figure 15a (Pl. **4**), made from several transverse sections, illustrates a fairly typical form of division of the ventral ramus. This shows a branch of a ventral ramus penetrating a myoseptum the width of one myomere above the ventral muscle border. In these sections, stained with Mallory's differential stain, the connective tissue may be readily discerned on either side of the penetrating branch. Upon its exit on the inner surface of the side muscles, the ascending visceral branch takes a course dorsad over the connective tissue covering of the muscles.

This branch runs for some distance inside a gonadial pouch. The descending branch passes downward over the inner surface of the most ventral myomere to the inner surface of the transverse muscles. Here it appears to lie between layers of connective tissue. Both the ascending and descending visceral rami show a faint bluish tinge in sections stained with Mallory's differential stain, due, perhaps, to the presence of a thin connective-tissue sheath.

The distribution of the branches of the *rami viscerales descendentes*, forming the inner abdominal plexus, has been well described by Fusari (**'89**), Heymans et van der Stricht (**'98**), and Dogiel (**: 02**). Fusari and Dogiel note a network of extremely fine threads connected with the coarser meshes of the inner abdominal plexus. Dogiel describes these threads as somewhat varicose. He also finds other fine branchlets given off from the coarser plexus, which run to the abdominal muscles, and break up into a great number of repeatedly dividing finer threadlets that possess small varicose thickenings. Dogiel is of the opinion that these threadlets weave about the transverse muscles in an exceptionally thick plexus, analogous to that which he has described for the "ring-muscle" of the mouth. He does not find the fibers described by Heymens et van der Stricht as passing directly from the coarser plexus to penetrate between the lamellae of the transverse muscle, where they terminate in swellings analogous to the endings of nerve fibers in smooth muscle. Dogiel notes small three-cornered nuclei in the angles formed by branchlets of the coarser abdominal plexus. Fusari finds no ganglion cells in connection with this plexus, but notes small nuclei at the knotted places of anastomosis. He designates the nerves of the inner abdominal plexus as sympathetic.

The finer plexus described by Fusari and Dogiel is clearly visible in certain of my methylene-blue preparations. In these preparations the threads of this finer plexus are less varicose than those figured by Dogiel (**: 02**, Fig. 14, 15). However, at rather infrequent intervals swellings resembling small nuclei may be seen. Many of these finer threads cross the interior faces of the coarser meshes, indicating that they lie closer to the epithelium covering the inner surface of the transverse muscles than does the main plexus. A few fine threads, however, cross the external surfaces of the larger meshes. The meshes of the finer plexus are generally elongated parallel to the long axis of the animal. A secondary plexus is also present in that portion of the abdominal region which lies between the transverse muscles of either side of the body, but its meshes do not appear to be as close

here as in other regions. In transverse sections stained with Mallory's differential stain, portions of the larger plexus may be seen lying on the inner surface of the transverse muscles, in connective tissue. Since many of the threads of the finer plexus are nearer the surface of the atrial cavity than those of the coarser plexus, they must be closely associated with the bases of the cells lining that cavity. In portions of my preparations the finer plexus is extremely complicated, and the meshes are not decidedly elongated. The meshes of the main inner abdominal plexus sometimes appear knotted in methylene-blue preparations, even though the knots are not differentiated in any way; but apparent nuclei are frequently present in the course of the nerve threads and at places of junction. Certain large meshes of the inner abdominal plexus send branches to the gonadial pouches (Pl. **3**, Fig. 13, *i.*), although the main supply for these structures is derived from the ramus visceralis ascendens. In Golgi preparations of Branchiostoma caribaeum, nerve threads were found scattered in the transverse muscles. It was impossible to determine their origin, but closely associated threads evidently did not all lie in the same focus. The greater number of these threads are arranged more or less nearly parallel to the long axis of the animal (Pl. **5**, Fig. 23).

Impregnation with methylene blue does not always produce the same results in the structural appearance of these visceral nerves. In some preparations the threads of the inner abdominal plexus appear granular, while in others distinct fibers can be traced. But notwithstanding these variations, the visceral nerves can always be distinguished from the cutaneous nerves by their rougher outlines.

Rami viscerales ascendentes.— Johannes Müller (**'41**, **'44**, p. 96), and Leuckart und Pagenstecher (**'58**) observed nerves passing to the branchial basket. Schneider (**'79**, p. 15) observed and figured sensory nerve branches penetrating to the interior at the ventral border of the side muscles. These branches ramify as they ascend on the surface of the abdominal cavity. He ascribes the function of the *vagus* to these nerves. Rohon (**'82**, p. 24) agrees with this interpretation. He also describes in some detail, the distribution of nerves in the ligamentum denticulatum, and in the branchiae. Fusari (**'89**, p. 130) describes the branchial nerve branch as ascending obliquely upward and forward after penetrating to the interior, finally joining the branchial apparatus by way of the ligamentum denticulatum. He states that only the more anterior nerves send branches to the branchiae and that the nerves of the tail also lack the "sympathetic" branch. It will be remembered that Fusari designates the nerves of the inner

abdominal plexus as sympathetic. According to Rohon, the branchial nerves end in tufts. Fusari (p. 133) differs from this, and states that they spread out in a membrane that covers and supports the branchial apparatus on its external surface. On this membrane the different nerve branches form a net of irregular meshes, which Fusari calls the nerve net of the branchiae (his Taf. VIII, Fig. 2). This figure probably illustrates a plexus formed by ascending visceral branches before they reach the branchial basket (compare Pl. **4**, Figs. 15, 16). Fusari probably saw a nerve net distributed over the gonadic pouches. Heymans et van der Stricht (**'98**, p. 41–44) describe in detail the branches of the rami viscerales ascendentes, and figure nerves in the ligamentum denticulatum, a small number of fibers on the branchial bars, and fibers on the cross bars, joining those running lengthwise of the primary and secondary bars (their Fig. 30). These authors also figure a plexus distributed along the median ventral portion of the branchial basket, and show a portion of a plexus covering the gonads. They never found nerve fibers in the interior of the branchiae. They note a plexus in the ligamentum denticulatum, parts of which resemble a longitudinal nerve trunk; but in their opinion an actual large longitudinal nerve does not usually exist, except as formed indistinctly by the anastomosis of different branches of the ascending visceral nerves. These authors are therefore inclined to deny the existence of a distinct vagus nerve. Other branches of the rami viscerales ascendentes were observed, which these writers (Pl. VIII, Fig. 26) regard as perhaps innervating the blood-vessels, digestive tube, and the parietal serous surface of the branchial cavity. Dogiel (**: 02**) presents figures of Golgi preparations showing numerous fibers on the primary and secondary branchial bars, and on the cross bars; also nerve cells of spindle-like or angular shape, previously undescribed, possessing from three to six processes, which gradually divide into a great number of twigs. Dogiel finds such cells in all parts of the branchial basket, and regards them as analagous to the sympathetic cells in the intestinal plexus of the "Neunauge" (river-lamprey). He also finds ascending visceral branches posterior to the branchial basket, which he is certain supply the intestinal canal (Fig. 17*a*, 17*b*, 18*a*, 18*b*, 19*a*, and 19*b*).

My own observations on the ascending visceral nerves and their branches were made chiefly from specimens of B. lanceolatum, only a few individuals of B. caribaeum proving favorable for study of these nerves. Dissected specimens previously impregnated with methylene blue were particularly useful in following the ramifications of the rami viscerales ascendentes. A study of these nerve branches is attended

with difficulties, since it is necessary to lift the pharynx wall to disclose the nerves on the internal surface of the side muscles, and in the ligamentum denticulatum, and this proceedure is more than likely to break the slender nerve threads passing to the ligamentum denticulatum and the branchial basket. The accompanying series of figures (Pl. **2**, Fig. 10; Pl. **3**, Figs. 13, 14; Pl. **4**, Figs. 15–21, Pl. **5**, Fig. 30) aims to illustrate the distribution of numerous branches of the rami viscerales ascendentes with as much clearness as these technical difficulties permit.

As has been noted, a visceral branch may bend around the ventral border of the side muscles, or penetrate a myoseptum further dorsalward. A dorsal nerve may possess two or more visceral branches, instead of one. Figures 13 and 15 (Pls. **3** and **4**) illustrate a condition of frequent occurrence, in which a branch of the ventral ramus penetrates to the interior through the myoseptum just dorsal to the most ventral myomere. In either case, division into a descending and an ascending visceral ramus takes place immediately after reaching the internal surface of the side muscles, the ascending branch running dorsalward, and the descending branch taking a general ventrad direction. In Figure 15 the descending ramus is small, but in Figure 13 its size is equal to that of the ascending ramus. Such variations in size are frequent, and when a branch is unusually small, compensation may ordinarily be noted in an adjoining nerve. The ramus visceralis ascendens gives off branches from either side as far as a main stem can be observed, but the origin of the more ventral of these branches is usually obscured by a gonadic pouch. When one of these pouches is empty (Fig. 13), the nerves which innervate its entire surface with an elaborate network may be readily seen arising either from the ascending visceral ramus, or its first branches, or from branches of the descending visceral ramus, or, finally, from meshes of the inner abdominal plexus. These nerve branches ramify to form a plexus which covers not only the more interior surface of the gonadial pouch, but also the surface adjoining the muscles. This plexus is extremely delicate and fine-meshed. The more exterior portion is continuous throughout the region of the gonadic pouches, the network arising from one nerve uniting with that which is in connection with an adjoining nerve. This network also joins the more dorsal fan-like branches of the rami viscerales ascendentes. This plexus is probably also continuous with that which is in connection with the intestinal canal, and it possibly extends beyond the anus. When the gonadic pouches are large and press against each other, the

plexus on their more interior surfaces appears continuous and the origin of branches from the visceral rami is largely hidden. Figure 13 (Pl. **3**) shows only the larger meshes of the plexus on the internal surface of a gonadic pouch, but the origin of these branches is illustrated. In Figure 10 (Pl. **2**) the finer meshes of this plexus are shown under greater magnification. In methylene-blue preparations the threads of the gonadic plexus often appear faintly granular, indicating that they impregnate in a manner similar to the nerves of the inner abdominal plexus. Heymans et van der Stricht (**'98**) find the "peritoneal" plexus less rich under the sexual glands, but in this they probably refer only to the larger meshes. These authors do not figure the origin of branches running to the gonadic pouches, and give no detailed account of their innervation. Dogiel (**: 02**) does not discuss the innervation of these organs.

The main stem of an ascending visceral ramus may usually be followed dorsad a distance equal to the width of one, two or even three myomeres from the ventral border of the side muscles, and then it breaks up into a large number of branches spreading out fan fashion over the inner surface of these muscles. In the anterior part of the body these fans are directed somewhat anteriad as well as dorsad (Pl. **4**, Fig. 15), but the branches of the more posterior ascending visceral rami take in general a dorsad direction. If the wall of the branchial basket is lifted to disclose these nerves, the numerous branches of a fan often appear broken off abruptly in the middle region of the side muscles, and the distal broken ends are most frequently bent away from the muscles as though lying in a membrane not closely attached to the side muscles. This is no doubt due to the entrance of these nerves into the ligamentum denticulatum. As may be noted in Figure 15, *c* and *d*, the branches of one fan anastomose with those of an adjoining fan, thus forming a continuous network along the inner surface of the side muscles, and in the ligamentum denticulatum. This plexus may or may not lie dorsal to the gonadic pouches, depending on the size of the pouches. As has been mentioned, these fans join nerve threads which innervate the sexual glands.

From the plexus formed by these fans, certain branches pass upward along the ligamentum denticulatum (Pl. **4**, Figs. 16, 17), while others ramify extensively in the "pocket" portions of this ligament. These nerves often present a fibrous appearance, as noted by Fusari; but this seems to depend on the time or manner of fixation. In other cases they appear dotted, with rough edges, the latter perhaps due to the deposit of a small quantity of coloring matter in the surrounding

tissue. The nerves to the branchial basket pass upward along the ligamentum denticulatum in numerous small threads, and enter the basket not far from its dorsal region of attachment. The nerves in the "pocket" portions of the ligament run more or less longitudinally (Fig. 17), while those in the portions attached to the primary bars take a nearly vertical direction. As may be noted in Figure 17, nerves pass to the primary and secondary bars from various portions of the ligament. Figures 14 (Pl. **3**) and 19 (Pl. **4**) show the nerve threadlets of the primary and secondary branchial bars, and the connecting threads passing along the cross-bars. These figures, drawn from methylene-blue preparations, show an interesting likeness to Dogiel's Figur 17*a*, taken from a Golgi preparation. In the course of treatment after impregnation with methylene blue, the branchial epithelium sloughs off in large measure, exposing the nerve threads. The nerves which pass dorsad along the primary and secondary bars lie at first on the more exterior (lateral) surface of the branchial basket. As the threads ascend, they gradually shift toward the interior, and a greater number of threads are often found on the more posterior portion of each primary bar. Near the top of the primary bar these threads pass into a plexus lying between any given bar, and the next posterior secondary bar (Fig. 20). It is quite possible that as many fibers ascend on the anterior side of the bars to join this dorsal plexus, but the specimens observed did not show as great a number. Nerves passing along the secondary bars join this dorsal plexus with a smaller number of threads. In the middle region of the branchial basket, and in its ventral portion, the nerve threads lie on its more external surface. The nerves of the primary bars are loosely placed, and form an irregularly meshed network, the threads of which are not noticeably arranged lengthwise of the bar (Figs. 17, 19). On the secondary bars (Pl. **3**, Fig. 14), on the contrary, the slightly knotted nerve threads form a plexus consisting of numerous long, longitudinally arranged threads, connected by short branches. This plexus appears to be rather smoothly drawn over the surface of the bar, in contrast to that of the primary bars. The knotted appearance of threads on the secondary bars may be an artificial condition. Nerve threads crossing from one bar to another along the cross-bars form a plexus over the latter in their course. At least, in the region dorsal to the ligamentum denticulatum fibers pass from primary to secondary bars at other points than the cross-bars, thus adding to the elaborateness of the branchial plexus.

I find a large number of the previously mentioned special nerve

cells, which were first described by Dogiel (: **02**) as connected with nerves of the branchial basket. Dogiel figures these cells as they appear in Golgi preparations, and states that in methylene-blue preparations he noted large round or oval nuclei on certain nerve branchlets of the branchial basket, which he identifies as the nuclei of the cells found in Golgi preparations of the branchial region. These "nuclei" are shown in his Figur 16, taken from a methylene-blue preparation. This figure shows a condition frequently present in my methylene-blue preparations. Comparatively smooth lines, darkly colored with methylene blue, run parallel to the branchial bars, but do not appear to be connected with the evident nerves of the pharynx. Possibly these may be blood vessels; at any rate the nerve cells (Figs. 19, 21) which are impregnated with methylene blue in my preparations are not connected with these lines, but with the branchial plexus. Figures 19, 21, and 30 show these cells in methylene-blue preparations, and in a Golgi preparation. They correspond in shape and number of processes to those described by Dogiel. The nuclei of the cells impregnated with methylene blue are often difficult to discern, as is frequently the case in other nerve cells impregnated more or less with methylene blue. This led to the suggestion that these bodies might themselves be nuclei. Upon measurement of these so-called cells, and those impregnated after the method of Golgi, it was found that the size in either preparation was practically the same, the bodies in methylene-blue preparations being if anything, a little rounder and fuller. In the Golgi preparation illustrated (Fig. 30) a lighter nucleus is plainly visible in the multipolar cell. These cells are present on both primary and secondary bars, and are present at least in the ventral and side regions of the pharynx, on its external surface. In the ventral region these cells are distributed in connection with the elaborate plexus which spreads over this portion of the branchial basket.

There are certain branches of the rami viscerales ascendentes which are connected with the fan-like branches of these rami, but apparently do not have the same ultimate destination (Fig. 15, *e*), since they are lost to view in the region between the muscles and the ligamentum denticulatum. Heymans et van der Stricht ('**98**) figure nerve branches of the ascending visceral rami (Pl. VIII, Fig. 26, *N D V.*), which they designate as digestive and vascular, but the destination of such branches has never been satisfactorily determined. It is certain, however, that further experiments with methylene-blue impregnation and other methods, will reveal many additional compli-

cations in the distribution of the visceral nerves of Amphioxus. The investigations up to the present time establish the fact that the visceral rami of the dorsal nerves are elaborately connected throughout the atrial region, and that multipolar nerve cells occur in connection with these visceral nerves.

E. Nerves Posterior to the Atriopore.

Dogiel (**: 02**) has described in detail the main cutaneous branches of these nerves, and illustrates the larger branches in his Figures 2 and 3. He notes the presence of "rami communicantes" connecting two adjacent ventral rami of dorsal nerves in their course over the side muscles of this region, but does not limit the occurrence of such connecting branches to the posterior portion of the body.

In the specimens which I have observed, the cutaneous branches in this region are more numerous than represented by previous authors. Rich plexuses are frequently present both between the dorsal rami and between the ventral rami of these nerves on the same side of the body (Pl. **6**, Figs. 34–36). These plexuses will be described in connection with the sensory nerve endings. A "ramus communicans" was frequently found in this region, as well as in other parts of the body. The elaborate branching of the last dorsal nerve of Branchiostoma caribaeum is illustrated in Figures 31 and 33 for the right and left sides respectively. This pair of nerves is exclusively cutaneous, and its branches are more numerous than the figures by previous authors indicate. The most posterior dorsal nerve is posterior to the last ventral root, and, according to Hatschek's interpretation of the relation of the nerves to the myomeres, belongs to the preceding myomere. He states that each dorsal nerve belongs to the myomere anterior to the myoseptum through which it passes, and that the ventral root innervating this myomere appertains to this dorsal root.

Little attention has been previously given to visceral branches in this region. Heymans et van der Stricht (**'98**, p. 43) note in Golgi preparations apparent nerve fibers on both the parietal and visceral mucosa faces posterior to the abdominal pore. They were unable to find the origin of these nerves, or to trace their course, but suggest that they may come from the "abdominal" visceral nerves (Hatschek), or, more probably, as an extension of "thoracic" longitudinal nerves into the "abdominal" cavity. These authors seem to apply the term "abdomen" to the region containing the intestinal canal. They also note in Golgi preparations nerve fibers supplying the surface of the

intestine, and (p. 44) branches from certain dorsal nerves (14th to 16th from the tail end) of the left side of the body supplying the anus. A branch from the right 13th (from the tail end) appears to have the same destination. Dogiel (**: 02**) states that the dorsal nerve pairs from XL, or XLI, to LIII, or LIV, inclusive, give off ascending visceral branches, which bend around the ventral border of the side muscles, or penetrate a myoseptum to reach the interior. Beginning with nerve XLI or XLII, these visceral branches divide into a great number of branchlets, which weave around the whole rectum reaching to the anus. A fine plexus is formed in the walls of the anus from exceedingly fine and slightly varicose branches of nerves LI, LII and LIII. Dogiel does not state that the nerves of one side are more concerned in the innervation of the anus than those of the other. It is uncertain whether or not he finds visceral branches from the nerves succeeding nerve LIV; his figures surely do not illustrate such branches. His Figures 19*a* and 19*b* show ramifications of the visceral branches of nerves XLII to XLVI inclusive. These extend on the inner surface of the side muscles from their ventral border a short distance dorsad. Both figures are drawn as seen from the exterior.

In the specimens I have examined, occasionally visceral branches were apparently lacking to certain dorsal nerves, but, as Dogiel has observed, the manner in which these penetrate to the interior may be such that a view from the exterior fails to give any evidence of their presence. Examination of a large number of specimens disclosed visceral branches to practically all the nerves of this region from XL to LXI, inclusive. However, visceral branches could never be discerned from the exterior for all of these nerves in a single specimen. In one case nearly all dorsal nerves as far as nerve LX showed visceral branches. I have never observed visceral branches in connection with nerves LXII, LXIII or LXIV (when 64 nerves are present). The innervation of the anus, which lies on the left side, is usually supplied by branches of *left* nerves LI–LIII, in both species. Variation occurs here, and it is probable that in Branchiostoma caribaeum more anterior nerves may occasionally innervate this structure. Frequently only two of the nerves mentioned above give off branches which weave about the anus. Right nerves LI, LII and LIII often show branches penetrating to the interior, but these could never be traced to the anus.

In methylene-blue preparations suitable for dissection, the intestine is rarely in favorable condition for study, although the main visceral nerve branches impregnate particularly well. Such preparations cut

along the dorsal median line, and laid open with the internal surfaces uppermost, proved very interesting when studied with the aid of strong light. Figure 32 (Pl. 6) is drawn from a specimen dissected after this fashion. It is evident from this figure that many more nerve branches penetrate to the interior than can be observed from an external view. These branches appear smooth and darkly stained for a short distance after they reach the interior, and their finer branches ramifying over the base of the ventral fin have a similar appearance. As the visceral branches ascend and ramify on the internal surfaces of the muscles, they are more lightly colored, and often flatten out into comparatively broad bands, which appear loosely confined. On the right side may be noted branches which probably supply the posterior extension of the atrium. The nerves ramifying over the base of the ventral fin are not to be confused with the cutaneous supply. The latter may be seen in a specimen dissected and examined in the manner just described, but they lie at a much lower focus (i. e. nearer the surface). A few nerves were observed forming a network over the inner surface of the side muscles posterior to the anus, thus accounting for at least a part of the visceral nerves observed from the exterior in this region. The nerve supply for the intestine probably reaches the latter through the membrane (mesentery) which holds it in place beneath the notochord. This corresponds to the manner in which a nerve supply reaches the pharynx. The presence of the post-atrial extension on the right side of the body in this region, may bring about modifications in the arrangement of the visceral nerves of that side. Certain abruptly ending branches, indicated at *c*, *c*, in Figure 32, may be the result of imperfect impregnation, or have a significance not yet determined. It seems certain that a network of nerve threads similar to that noted in the branchial region spreads over the internal surfaces of the muscles posterior to the atriopore.

In certain transverse sections of the tail region, stained with Mallory's differential stain, a pathological condition was evident, involving nearly all the structures of one myomere, and present to a slight extent in an adjoining one.

Considerable variation was noted in the total number of dorsal nerve pairs in each species. In Branchiostoma lanceolatum the number counted most frequently was 63; while in B. caribaeum the number appeared to be between 56 and 60.

F. Spinal Ganglia.

Dogiel (: **02**) found special structures in connection with the dorsal nerves of Amphioxus which he interprets as spinal ganglia. These bodies appeared in specimens immersed from 3–6 hours in a dark blue or violet mixture either of a 1% methylene blue, or of a saturated solution of toluidin blue, in normal salt solution. They could also be seen in gold-chloride preparations when the lemon-juice and formic-acid method was used. These so-called ganglia consist of groups of from 3 to 7 round, oval or pear-shaped elements, situated at the place of exit of each dorsal nerve from the myoseptum, or near the proximal end of the dorsal or ventral ramus where it gives off small branches; in addition to these, similar, though smaller, bodies may be found even as distant as the ventral border of the trunk muscles. These structures are illustrated in Dogiel's Figures 20, 21, 22*a*, 22*b*, 22*c*, 22*d*, and 23.

By using Dogiel's methods I was able to observe these structures in methylene-blue and in gold-chloride preparations, and also found them in specimens fixed in weak osmic acid and stained with picro-carmine. They appeared not only at the places indicated by Dogiel, but by using strong light for study of the preparations, they were found at almost any point in the course of the dorsal nerves. In methylene-blue preparations the size and number increased with the length of immersion. These bodies were of varying size, in some cases quite large, while by using the higher magnifications on portions of tissue mounted under a cover-glass, similar structures of minute size could be seen in connection with the smaller nerve branches. Strong light revealed great numbers of these structures in connection with the nerves of the thicker parts of the body. The methods mentioned above always cause a marked enlargement of the ampulla-like dilation at the posterior end of the neural tube, and a general increase of size in the tube itself. These facts led to doubts as to the reliability of such methods, and numerous experiments were made in the spring of 1905 to determine the nature of these bodies. Dogiel's methylene-blue method, employing normal salt solution instead of sea-water, causes general swelling of the tissues, and in some cases death occurs, followed by certain post-mortem changes, before these bodies appear. In material "fixed" in weak osmic acid and stained with picro-carmine these structures were frequently present, or, if not, I was able at will to make them appear by pressing lightly on the cover-glass over the mounted specimen. In some cases the pigment of the neural tube had

passed out into the swellings in the course of treatment with chemicals, or upon pressure, and contrasted brilliantly with the light stain. An excellent illusion was sometimes produced by the presence of well stained nuclei, which are either pushed out from the cord, or are sheath nuclei. The weak osmic acid has little effect on tissues beneath the surface, and the subsequent washing in water causes actual maceration. This would account for all manner of artifacts. The specimens treated according to the gold-chloride method mentioned above, show general distortion, and many structures are so displaced as to make such preparations quite unreliable for study. These bodies, if present in methylene-blue preparations, will persist when the preparations are preserved in the mixture of glycerine and ammonium picrate; but when fixed in ammonium molybdate and *dehydrated* they usually disappear. Similar structures were never found in well preserved and sectioned material. There seems, therefore, but little doubt that these so-called "ganglia" are artifacts. Since writing the above account, Johnston's (**: 05**) paper was noted, in which he also concludes that these structures are of artificial formation. Johnston finds a small proportion of the ganglion cells of Amphioxus in the nerve cord, and the remainder in the dorsal roots, located as far as, and beyond, the place of division into dorsal and ventral rami.

G. Structure of the Dorsal Nerves.

In methylene-blue preparations of the dorsal nerves, darkly stained fibers can often be traced from the neural tube, or place of exit of the nerve, through the myoseptum to the ventral border of the lateral muscle. These fibers are more or less separated from one another, and are not of equal size. Usually a single fiber cannot be traced for this entire distance. Fibers can frequently be discerned in the rami cutanei ventrales which give these branches a darkly colored appearance. I have often traced one, two or three fibers into the common basal trunk of the descending and ascending visceral branches, but was unable to trace distinct fibers into either of those branches. As has been noted, however, these nerves sometimes present a fibrous appearance. Figures 40 (Pl. **7**) and 22 (Pl. **5**) show fibers of at least two sizes entering the dorsal nerve roots, the smaller fibers being by far the most numerous. This agrees with the observations of Johnston (**: 05**). The different structural appearance of the visceral nerves has already been noted. This difference is particularly evident in

methylene-blue preparations, and may be observed in all branches of dorsal nerves innervating deep-lying structures. It is entirely possible that structural distinctions will be established between the visceral nerves to different organs.

H. Sensory Endings of Dorsal Nerves.

This subject has long attracted investigators, and its study has brought forth a great variety of opinion and suggestion. Quatrefages ('**45**) described peripheral nerves ending in small ovoid bodies (the corpuscles of Quatrefages), which he suggested represent special mucous organs. Kowalewsky ('**67**) believed that the sensory nerves end in epithelial cells. Owsjannikow ('**68**) occasionally saw what appeared to be a nerve fiber connecting with a cylindrical epithelial cell. He also noted what he calls a nerve-net, lying in the deepest layer of the skin, a region well supplied with connective tissue. Reichert ('**70**) describes certain cells in the epithelial layer of the skin, which bear a spine-like external process. He designates these cells as "thorn" cells. Langerhans ('**76**) found no branches uniting two dorsal nerves, or any exchange of fibers. All dorsal nerves branch in simple tree fashion. He believes that nerve plexuses are lacking in all parts of the body, except at the mouth border. He found no vestige of a fine end plexus such as Marcusen describes, holding that Marcusen was deceived by connective-tissue fibrillae. Langerhans describes special small cells lying irregularly between the cylindrical epithelial cells of the skin. These special cells have a small body and large oval nucleus; they lack a cuticula (limiting or basement membrane), and often possess a thread-like process at the internal end. On the external surface each cell bears a long, stiff hair, which sometimes has a thickened base. These special cells are particularly numerous in the head region, but may be found on all parts of the body. The hairs may be seen on living animals, but never show motion. The finer dermal nerve branches pass through small canals in the so-called limiting membrane of the skin, each of which is located where two small fissures in the membrane cross each other, usually at right angles. After a short sub-epithelial course, these fibers unite with hair-bearing cells, which Langerhans declares to be the endings of the cutaneous nerves. These nerves are not in relation with the ordinary epithelial cells, in fact, there are not enough nerve branchlets to permit union with each such cell.

Rohon ('**82**) states that the greater number of nerve branchlets run

out into the cutis and end in tufts. Fusari (**'89**) says that the cutaneous nerves divide into fine branches immediately beneath the cuticula (the limiting membrane of Langerhans), and that anastomoses are frequent in the skin of the ventral region, but rare on the sides and dorsum. The branches in the ventral region pass into fine free-ending fibrillae, or before ending show a spindle-shaped, nucleated enlargement. In all other regions (with the exception of the mouth) nerves, penetrating the cuticula, appear to terminate after a short course in free endings, though they can be traced to the bases of epithelial cells. Fusari is uncertain, however, whether there is any connection between these nerve fibers and the cells. He saw the sensory cells of Langerhans, and though inclined to his views, contends that a connection with the nerves is not demonstrated. He believes that the description of the nerve-net given by Marcusen is based on a peritoneal rather than a dermal plexus. Heymans et van der Stricht (**'98**, p. 33) regard the so-called sense-cells of Langerhans as merely compressed and flattened "cyclinder" cells, bearing no special relation to the nerves. These authors saw branches of a dorsal nerve form among themselves a plexus on the border of the fins; but they never found the plexuses mentioned by Fusari as frequent in the ventral region. According to these writers the nerve fibers ramify under the cutaneous epithelium, and terminate between, and perhaps within, ordinary epithelial cells. They found no special sense-organs in the skin, in connection with nerve fibers.

Retzius (**'98**) is convinced that cutaneous fibers end free at the bases of epidermal cells. He saw two kinds of cells in the epithelium, one smaller than the other, but neither bore a thread- or spine-like process. He found no direct connection between these cells and nerve fibers, nor could he discover any true peripheral sense-cells connected with nerves. Dogiel (**: 02**) states that the "Nervi cutanei dorsales (laterales et ventrales)" are pure sensory branches without any motor fibers. These gradually divide in their course to the superficial layers of the skin, continually giving off branches of various lengths and thicknesses, which anastomose with similar neighboring branchlets to form a more or less elaborate plexus, his "Grundgeflecht." This plexus is especially plain, he says, in the skin of the ventral region. His further description is as follows: The branches from this plexus ultimately reach the homogeneous layer of the skin immediately beneath the epithelium, and can be observed in good preparations to pass through small canals in this layer. Each then divides into three or four or more fine branchlets, which spread out radially. These

branchlets, which lie immediately beneath the epithelium, in turn divide repeatedly in this region, giving off fine, varicose threads, which form a close-meshed plexus. This is designated by Dogiel as the "sub-epithelial plexus." From this plexus fine threadlets penetrate between the epithelial cells, and there give off side branchlets, which twist about these cells and end between them; these threads were followed almost to the outer surface of the skin. Dogiel found such endings in the rostrum, in the middle region of the body, and in the tail. But in addition to these free nerve endings in the epithelium, Dogiel also saw in the epithelium what he regards as peripheral nerve cells; these occur in the rostrum, the tentacles, and the "head" region. He believes that they are scattered over the entire skin area. These cells have a spindle-shaped body, with a peripheral and a central process, and a large nucleus, nearly filling the thick part of the cell. The peripheral process is a short, thick cylinder or rod, which reaches close to the outer surface of the epithelium, and its end is either blunt, or somewhat pointed, but it never extends in the form of a thread beyond the free surface of the skin. The central process runs perpendicularly or obliquely toward the interior, and could occasionally be seen passing through the homogeneous layer of the skin and uniting with a nerve branchlet. Dogiel's Figur 28 presents a portion of the "sub-epithelial plexus" and its branches in the ventral region of the body, in connection with the well-known plexus formed from branches of the rami cutanei ventrales. His figures 29 and 30 show peripheral nerve cells in the skin of the rostrum and head. Dogiel also illustrates nerve-endings in the tentacles of the mouth.

In my own study of the sensory nerve terminations in Amphioxus a great number of methods were employed, including methylene-blue impregnation, the methods of Golgi, the various gold-chloride methods, picro-carmine staining, and sections of material fixed and stained in a variety of ways. As has been noted, the extensive arborescent appearance of the cutaneous branches of the dorsal nerves may be readily observed in methylene-blue preparations studied with the aid of strong light (Pl. **6**, Fig. 34; Pl. **7**, Figs. 37, 39). The arrangement of the sensory endings must depend largely on the distribution of the finer terminal nerve branches. The extreme readiness with which Amphioxus reacts to tactile stimuli applied to any part of the body, indicates a rich supply of cutaneous nerves. The sensory nerve branches reaching to the epithelial layer of the skin are most readily followed in methylene-blue preparations. The best impregnations of these nerves were obtained by immersion of the specimens from $1\frac{1}{4}$

to $1\frac{3}{4}$ hours. The subsequent exposure to air varied from 15 to 40 minutes. The specimens were fixed in ammonium picrate to which a little osmic acid had been added, or in ammonium molybdate and osmic acid. Direct sunlight, a Welsbach lamp, or a Nernst lamp were used in studying whole specimens.

The distal ends of the finer cutaneous nerve branchlets are often noticeably directed toward the exterior (Pl. **7**, Fig. 37). By focusing on the plane in which the exterior ends of these nerve branchlets lie, it may be observed that the surface of the cuticula is broken by numerous small clefts crossing each other either at right angles, or in the form of an X. By focusing downward upon a dark spot at the place of crossing, it can be determined that this spot is the end of a nerve branchlet, which can be followed to its place of union with one of the larger branches. These numerous small, exteriorly directed branchlets for the most part, therefore, penetrate the sub-epithelial so-called cuticula (better basement membrane) through the small canals first described by Langerhans. These small branchlets are more numerous than either Heymans et van der Stricht (**'98**), or Dogiel (**: 02**) have figured. They were not always evident, and it was frequently impossible to demonstrate a basement membrane, which seems to indicate that this structure sometimes sloughs off with the epithelium. Figures 34 (Pl. **6**) and 39 (Pl. **7**), which show these exteriorly directed small branchlets in considerable numbers, also show other characteristic cutaneous nerve branches. I refer to large branches, lying close to the muscles, which may be noted connecting the branches of two or more dorsal nerves in their course over the side muscles (see also Pl. **6**, Fig. 36, p_1 and Pl. **7**, Fig. 38, p_1). Beside these connecting branches, there are, lying at a higher focus, finer plexuses, which connect the small, exteriorly directed branchlets. These finer plexuses may also be seen in Figures 35, p_2 and 38, p_2. In methylene-blue preparations this finer plexus is usually the most superficial nervous structure visible. The branches of the deeper plexus are usually of considerable size, and fewer in number than those of the more exterior plexus, and their meshes are larger than those of the outer plexus. As has been noted, the deeper plexus lies close to the muscles, and its branches may even run beneath other large branches of the dorsal nerves. This plexus between the ventral rami of dorsal nerves was found distributed over the side muscles in nearly all parts of the body. The finer plexus is composed of slender threads, forming close meshes; it was found in the regions where there is a coarser plexus, and also in the ventral fin, and between the dorsal rami of dorsal nerves. The

nerve threads in either plexus always appear smooth, showing no swellings of any kind. Figure 34 (Pl. **6**) shows at *c* (near the upper margin of the figure) a cell which is apparently associated with a fine nerve plexus.

These plexuses were found in nearly all specimens, of both species, impregnated in the manner previously described. The specimens retaining considerable epithelium were quite unfavorable for study; on the other hand, with the sloughing off of the epithelium, portions of the superficial plexuses were undoubtedly lost. A record, given below, was made of the occurrence of these plexuses, indicating the species, the side of the body, and the number of the nerve with which they were associated. In nearly every case, portions of both the coarser and the finer plexus were present.

	Species.	Side of Body.	Number of Nerve.
1.	B. lanceolatum	left	IX
2.	" "	"	XI, XII } same individual
		right	" " }
3.	" "	left	XV, XVI, XVII
4.	" "	right	XXI, XXII } same individual
		left	XXVI, XXVII }
5.	" "	right	XXVII
6.	B. caribaeum	"	XXIX, XXX
7.	B. lanceolatum	left	XXXII
8.	" "	right	XXXV, XXXVI, XXXVII
9.	B. caribaeum	left	" " "
10.	B. lanceolatum	"	XXXVII, XXXVIII
11.	" "	"	XLI, XLII, XLIII, XLIV
12.	" "	"	XLII, XLIII, XLIV
13.	" "	right	XLVI, XLVII, XLVIII
14.	" "	"	LIV, LV
15.	" "	"	LIII, LIV, LV, LVI, LVII
16.	" "	"	LIV, LV, LVI

It is evident from this record, that these plexuses cannot be looked upon as localized structures, or of infrequent occurrence. The record shows no plexuses (those of the mouth border are not included) in the rostrum, buccal region, or at the extreme posterior end of the animal. This may be due to lack of impregnation, or these regions may show differences in this respect. Figures 34, 35, 36 (Pl. **6**) and 38, 39, 41 (Pl. **7**) present the first published views of such cutaneous plexuses distributed over the side muscles, either *in situ* or otherwise.

The plexuses shown in Figures 34, 38, and 41 — formed by the breaking up, for a short distance, of the main stem of the ventral ramus of a dorsal nerve into a number of anastomosing branches,— are of frequent occurrence, but do not appear to have been noted by earlier authors. Such plexuses may have a morphological significance, or they may be pathological, the result of injury or disease. A simpler form of division of the ventral ramus is often present, in which the main stem divides into two branches of nearly equal size, which unite again after a short course. These modifications in the ventral ramus often serve to attract attention to fine plexuses in connection with them. "Rami communicantes" were often noted in both species.

In the figures showing plexuses between the dorsal nerves, branches may often be noted, in connection with at least the coarser plexus, which penetrate the myosepta toward the interior of the muscle (*a*, Figs. 34, 36, Pl. **6**). The destination of these branches is uncertain.

The absence, in methylene-blue preparations, of any great number of branches arising from the finer plexuses, is of interest. It may, perhaps, be accounted for by the fact that such thick preparations are not favorable for disclosing fine threadlets; higher magnifications of course cannot be used. Gold-chloride preparations of the skin were made to supplement those on which are based the foregoing account of terminal branches. The method of Ranvier was used, also that of Hardesty, in which impregnation with gold chloride follows fixation with 10% formol. The latter method gives accurate fixation as a rule, and the finer cutaneous nerve branches often adhere to the skin, when it is stripped off, instead of remaining attached to the muscles. Such preparations can be studied with the aid of the higher magnifications; but the epithelial cells always obscure the nerves more than is desirable. Figure 24 (Pl. **5**) is drawn from a gold-chloride preparation of material fixed in formol. This figure shows the internal surface of skin stripped from the dorsal fin toward the posterior end of the animal. A few epithelial cells are outlined (at *e*) to show their relative size, and special cells, to be described later, are indicated at *g* (upper part of figure). The fine threads apparently running out from the larger nerve branch are the most delicate threads so far noted in connection with the cutaneous branches of dorsal nerves; and the star-like places of anstomosis (*s*) of these threads remind one of those described by Dogiel. Apparent cells or nuclei (*c*) may be noted in connection with these threads, as the latter run to the special cells (indicated at *g*) situated between the epithelial cells of the skin. Unfortunately, however, connective-tissue threads stain very well with gold chloride,

and therefore may lead to much confusion. In the ventral region, where the tissues are transparent, connective-tissue threads may be readily discerned in gold-chloride preparations, weaving about the nerves, and running out from them in various directions like guy-ropes. These threads thus furnish an excellent pitfall for the observer. Where exceedingly fine nerve branchlets and connective-tissue threads are associated with each other, one may well hesitate to decide between them. The bodies (*c*) connected with the fine threadlets shown in Figure 24, all lie in the same plane with the threadlets, whereas the epithelial cells and special cells (*g*) are more superficial, being immediately exterior to these threadlets. The latter may be terminal nerve branches, interweaving at the bases of the epithelial cells, and sending branches between them, but evidence furnished by some other method is needed for corroboration. If these threadlets are nerves, they are probably connected with the finer plexus noted in methylene-blue preparations.

The special cells (*g*) previously mentioned are shown in Figure 42 (Pl. **7**) under greater magnification. This figure illustrates the external surface of skin overlying the side muscles near the dorsal fin. This preparation was impregnated with gold chloride after Ranvier's method. In preparations of this kind the ordinary epithelial cells are light purple in color, while certain differentiated cells lying between them appear red, are somewhat smaller, and are oval or circular on their external faces instead of polygonal. One or two slightly modified epithelial cells (*s*) are usually associated with each special cell. These accompanying cells assume a darker purple color than the ordinary epithelial cells. The special cells (*g*) and the modified epithelial cells (*s*) were both present in certain methylene-blue preparations, distributed in the rostrum and anterior portion of the body as far as nerve XVI. Unfortunately, favorable preparations of the skin of more posterior portions of the body were lacking. In these methylene-blue preparations the special cells assume a dark blue color, while the modified epithelial cells are only slightly darker colored than the ordinary epithelial cells, and present a granular appearance.

Changes in focus show that the diameter of these special cells is slightly less at the surface than at a short distance toward the interior. The most striking feature, however, of these cells is a rather long, stiff hair-like structure attached to the outer surface of each cell. In gold-chloride preparations this hair-like structure is colored black, or a very dark purple. In methylene-blue impregnations it appears dark blue. As shown in Figure 42 (Pl. **7**), this "hair" is thicker at its

distal than at its proximal end, and often bears a knob-like termination. Two apparent points of attachment to the cell are frequently visible; one of these occurs at almost any point on the outer surface of the cell, while the other is at one side of the cell, or perhaps between two cells. While these special cells may possibly correspond to the "thorn" cells (Stachelzellen) noted by Reichert (**'70,** p. 756), this stiff hair is much too long for such a comparison, nor does it resemble a spine, because of its thickened distal end; neither does it resemble any terminal nervous structure. One interesting feature is the varying length of such "hairs," and their absence from many of the special cells. The "hairs" were nearly always comparatively short in methylene-blue preparations. I suggest that these special cells may be gland cells, and that the "hairs" are hardened exudations adhering to the outer surface. Glands certainly exist in the skin of Amphioxus, and probably receive a nerve supply from the cutaneous plexuses. Attempts were made on hardened material to color the "hairs" with special stains for glands, but none of the material was properly fixed to make such tests of value. If these peculiar cells are gland cells, it is still uncertain whether sensory cells are present in the epithelium of all parts of the body.

Sensory cells are clearly present in the tentacles, and on the mouth border, as described by Dogiel (: **02**, p. 192–195; Fig. 10, 31, 32). These cells impregnated successfully in certain of my methylene-blue preparations, and nerve fibers could be traced in the tentacles to the region of these cells. I was unable, however, to determine absolutely the connection between the two which in all probability exists in the form already shown by Dogiel. The long distal process of the end-cells in the papillae of the tentacles, figured by Dogiel was clearly evident in my preparations.

Ventral Nerves.

Owsjannikow (**'68**) describes nerve roots arising from the spinal cord at different levels. Although Stieda (**'73**) thought it probable that this author saw ventral nerve roots, he himself was the first to describe the ventral roots clearly; he notes that they do not lie in the cross-section plane of the dorsal roots, and he found no actual union between them and the dorsal roots. Langerhans (**'76**) saw only dorsal roots, and regarded them as carrying motor fibers. Schneider (**'79**) described each ventral nerve root as arising by several processes

(each leaving the nerve cord by a separate orifice), which unite into a flat bundle; this soon spreads out, a small portion going dorsad, and a larger part ventrad. When the fibers reach the muscle layer they bend posteriorly and coalesce with the free margin of the muscle-plate. As the fibers approach the border of the muscle-plate, they become cross-striated. Schneider proposed the theory that the real motor nerves extend outward only as far as the sheath of the spinal cord, and that each muscle-plate sends a process to the spinal cord and receives its innervation there. Balfour (**'80**) denies the presence of motor roots. Rohon (**'82**, p. 14, 54) states that the ventral roots arise from multipolar ganglion cells, in part pigmented, which lie on both sides of, and in part ventral to, the central canal of the cord. He finds no morphological relation between dorsal and ventral roots outside the spinal cord and is entirely in the dark as to how the motor nerves end. Rohde (**'88**) thinks, with Schneider, that the motor fibers are probably in part direct processes of the muscle fibrillae; but he is uncertain whether there is a direct connection between the motor fibers and the nerve elements of the neural tube. Fibers in the ventral roots were followed centripetally to a delicate membrane on the inner side of the corda sheath, where they were believed to divide into very fine fibers, which, however, could not be traced in the spinal cord. Fusari (**'89**) finds, as did Schneider and Rohde, most of the ventral-root fibers cross-striated; but some of them are not striated, exhibit enlargements, and stain black by the Golgi method. Retzius (**'91**) notes in each ventral root an anterior division composed only of varicose fibers, and a posterior, somewhat smaller division carrying fibers mostly without varicosities. Each fiber of the posterior portion bears an oval body a short distance after its exit from the spinal cord. Retzius is unable to decide whether these are sheath nuclei, or of some other nature. Toward the spinal cord motor nerve fibers were followed singly into the outer layer of the cord, where they enter a low, granular mound; but beyond this they cannot be followed. In these mounds the fibers often bend in hook-fashion. Retzius thinks that the connection of motor fibers with central ganglion cells, which he does not doubt, cannot be very direct. Fibers occasionally divide dichotomously, but there are no special nerve endings. Especially characteristic of these fibers is their varicose-granular condition. Owing to the abundance and transverse elongation of the granules, they give a cross-striated appearance to the fibers, probably the cause of Schneider's erroneous views, for these are true nerve fibers, and not of muscular nature. He is inclined to accept Rohde's suggestion that

the *transverse* muscles are innervated by fibers carried by the dorsal roots.

Heymans et van der Stricht (**'98**) believe that the presence of a pair of dorsal nerves behind the last ventral pair indicates the disappearance, or non-appearance, of a posterior myotome, and on this basis regard each dorsal root as corresponding to the following, not the preceding (as Hatschek states), ventral root. The division of a root into dorsal and ventral portions (Retzius) is based on an optical illusion, or on the effect produced by sections. The motor fibers, studied largely on Golgi preparations, penetrate between muscle plates and reach the peripheral zone of the myotome; they sometimes branch. Both ascending and descending fibers show varicosities, consisting of regular and of irregular swellings. The regular swellings are probably nuclei, but those of irregular occurrence are artifacts. Motor fibers show definite terminal bodies, which are flattened and conical, of spatulate form; they are perhaps to be considered as motor plates of the cylindrical muscle fiber, upon which the terminal nerve fiber inserts itself, not perpendicularly, but laterally. From the number of these terminal plates the authors believe that each muscle fiber is innervated. The nerve fibers of the ventral roots are non-medullated, and thicker than those of the dorsal roots. The striated fibers in ventral roots are not nerve fibers, but muscle fibers, deflected from their usual course.

Dogiel (**: 02**) impregnated motor fibers and end-plates with methylene blue. He finds that the typical motor nerve ending is not a plate, but a flattened cone, the basal surface of which lies on the surface of a *muscle* plate. The cones appear larger in Golgi preparations than in those impregnated with methylene-blue, because in the former case silver is deposited to some extent in the muscle as well as in the nerve. Dogiel finds motor fibers penetrating (centripetally) the ventral part of the nerve cord in more or less thick bundles. These fibers arch backward, and can be followed for some distance. Certain fibers bend downward under the central canal, either from right to left, or vice versa, and so constitute a kind of commissural fiber. Dogiel could not follow fibers to cells, but states that they do not divide into fine threads, nor form a net on entering the nerve cord. Although he says that few varicosities are present in methylene-blue preparations, his Figures 43, 44*a*, and 44*b*, show frequent swellings in the motor nerve fibers. Repeated branchings of the motor fibers in their course toward the periphery are seen in his Figure 41.

In my own study the most successful impregnations of motor fibers

were obtained by use of the rapid methods of Golgi, and by treatment with gold chloride after fixation with 10% formol. The methods of Golgi and their modifications have produced such varying results in the appearance of nerve fibers in vertebrates generally, that a great number of variations in length of time, temperature, etc., were employed in order to test, if possible, the reliability of the impregnations obtained. Several hundred specimens were subjected to these various modifications of the methods of Golgi, and enough specimens have been sectioned and examined to establish some definite facts. In general it was found that all animals must be cut into two or more pieces, depending on their size; since whole specimens, beside impregnating unsatisfactorily, usually show numerous artifacts. It is absolutely essential that the fluids employed be used in large quantities, and slight warmth was found advantageous in bringing about impregnation. A liberal amount of the silver nitrate should be used and changed at least once. The rapid methods were by far the most satisfactory, as shown by the greater number of fibers impregnated, and the freedom from artifacts. Dogiel states that varicosities in the motor fibers are constantly present in Golgi preparations. The accompanying figures (Pl. **5**, Figs. 25–29; Pl. **8**, Figs. 43–50) show that such is not the case. The difficulty in securing impregnations of motor fibers after they enter the neural tube suggests that their chemical composition may differ in that region. Modifications in the methods employed may hereafter bring results in this case, as in others.

The impregnations with gold chloride after fixation with 10% formol are comparatively free from distortion and artifacts, and are probably reliable as a basis for comparison. The impregnation of motor fibers and endings is somewhat uncertain with this method, but when successful the preparations are very satisfactory. The nerve fibers are not strikingly differentiated, but are favorable for study on account of their accurate fixation. These preparations show a tendency to fade after a considerable length of time. I did not succeed by this method in impregnating fibers distinctly as they enter the neural tube, but variations in the method may produce better results.

Strong light was particularly useful in studying the thick sections necessary for tracing the nerve fibers. The shrinkage in the surrounding tissues often causes wrinkling in fibers impregnated by the Golgi method (Pl. **8**, Figs. 46, 47). These fibers also show a tendency to break, but the artificial nature of such breaks is usually evident.

The ventral nerves were studied in both species of Branchiostoma, and no differences were found which could not be readily explained by the uncertainty of the methods employed. The nerve fibers of the ventral nerves show such uniform smoothness in good preparations that it seems doubtful if any actual "varicosities" exist. The numerous smooth fibers illustrated in Figures 28, 29 (Pl. **5**), and 46 (Pl. **8**), and the uniform smoothness of fibers impregnated with gold chloride, certainly give grounds for such a conclusion. The small swellings sometimes present in my Golgi preparations are of irregular occurrence, and are usually found in whole specimens, or those impregnated according to the slow methods. It is true that occasionally structures resembling bipolar ganglion cells (Pl. **8**, Fig. 50) are to be found toward the proximal ends of fibers in the ventral nerves, but one hesitates to regard these structures as cells when their presence here would indicate a marked variation from the typical condition. In Figure 50 an apparent nucleus is present in the cell-like body attached to the nerve fiber; but every investigator who has used the methods of Golgi knows what bewildering artifacts can be produced.

According to my preparations the motor fibers of Amphioxus leave the inner border of the spinal cord sheath (Fig. 25, Pl. **5**) as rather slender, smooth fibers, apparently differing somewhat in size. These fibers are often curved or wrinkled in the sheath, and frequently bend sharply as they leave the covering of the neural tube. They are continued to the muscle border (Figs. 25, 26) as comparatively smooth threads, changing little in caliber. The spreading out of the ventral nerves has been described so often that it need not be taken up here. Upon reaching the side muscles certain motor fibers increase in size, while others do not change, depending apparently on their distance from the muscle fibers which they innervate. As a fiber approaches its distal end, it gradually thickens, and finally ends with the peculiar plate- or cone-like structure, first noted by Heymans et van der Stricht (**'98**). In contrast to the observations of Dogiel (**: 02**), little branching is evident in the course of these fibers. Figures 28, 29 (Pl. **5**) and 46 (Pl. **8**), which show a large number of motor fibers, do not reveal branching. In Figures 28 (Pl. **5**) and 43 (Pl. **8**) single fibers may be traced from near the internal border of the muscles to a short distance from its exterior border and in one case (Fig. 43) to the termination of the fiber. These long, single fibers are significantly frequent in various parts of the myotome. Branching certainly occurs (Pl. **8**, Figs. 48, 49), but my preparations do not reveal the extensive division in the region of the muscles described by Dogiel.

It may be noted in this connection that a greater number of nerve fibers are impregnated in the region of the muscles than in the neighborhood of the neural tube. This might be due to any one of several conditions; e. g., a fiber may change in character before it reaches the neural tube, thus presenting for impregnation different conditions in its two parts; or branching may take place more freely than my specimens indicate. Perhaps the mechanical tension on the nerves, between their exit from the neural tube and the nearest point of the muscle, due to the action of reagents, may destroy, or render unsuitable for impregnation, that part of the nerve.

The motor endings shown in Figures 27, 28 (Pl. 5) and 43–45 (Pl. 8) are probably fairly typical. They agree in size and form with those in gold-chloride preparations, and the surrounding muscle does not show the deposit of silver sometimes evident in material impregnated by the Golgi method. These endings were found in nearly all portions of the side muscles except the region adjoining the skin. They are present in considerable numbers a short distance from the exterior. Many endings were found dorsal to the neural tube, not far from the dorsal fin. I was unable to trace motor fibers into the neural tube beyond its sheath. No preparations of any kind showed motor fibers continuing in the neural tube in the manner figured by Dogiel in his Figur 45. In certain preparations, not cut in a true frontal plane, fibers appeared to end some distance inside the sheath of the neural tube, but on further study this proved to be an illusion, caused by the direction of the section. I am inclined to believe that the true course of motor fibers in the neural tube is as yet undiscovered.

Bibliography.

Balfour, F. M.
'80. On the Spinal Nerves of Amphioxus. Quart. Jour. Micr. Sci., Vol. 20, pp. 90–91.

Dogiel, A. S.
: 02. Das periphere Nervensystem des Amphioxus (Branchiostoma lanceolatum). Anat. Hefte, Heft 66, pp. 143–211, Taf. 12–29.

Edinger, L.
: 06. Einges vom "Gehirn" des Amphioxus. Anat. Anz., Bd. 28, pp. 417–428.

Fusari, R.
'89. Beitrag zum Studium des peripherischen Nervensystems von Amphioxus lanceolatus. Internat. Monatsschr. f. Anat. u. Physiol., Bd. 6, pp. 120–140, Taf. 7, 8.

Goodrich, E. S.
: 02. On the Structure of the Excretory Organs of Amphioxus. Part I., Quart. Jour. Micr. Sci., Vol. 45, pp. 493–501, pl. 27.

Goodsir, J.
'41. On the Anatomy of Amphioxus lanceolatus; Lancelet, Yarrell. Trans. Roy. Soc. Edinb., Vol. 15, Part I., pp. 247–263, pl. 4, 5. *Also in* The Anatomical Memoirs of John Goodsir, edited by William Turner, etc., Vol. I., Edinburgh, 1868, pp. 371–393, pl. 1, 2.

Hardesty, I.
: 02. Neurological Technique, etc. xii+183 p. Chicago and London.

Hatschek, B.
'81. Studien über Entwicklung des Amphioxus. Arbeit. Zool. Inst. Univ. Wien, Tom. 4, pp. 1–88, Taf. 1–9.

Hatschek, B.
'92. Die Metamerie des Amphioxus und des Ammocoetes. Verhandl. Anat. Gesell., Sechste Versamml. Anat. Anz., Jahrg. 7, Ergänzungsheft, pp. 136–162.

Hesse, R.
'98. Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. IV. Die Sehorgane des Amphioxus. Zeit. f. wiss. Zool., Bd. 63, pp. 456–464, Taf. 24.

Heymans, J. F., et van der Stricht, O.

'98. Sur le système nerveux de l'Amphioxus et en particulier sur la constitution et la genèse des racines sensibles. Mém. couron. et mém. sav. étrang. Acad. Roy. sciences, lettres et beux-arts Belgique, Tom. 56, mém. 3, 74 pp., 13 pl.

Johnston, J. B.

: 05. The Cranial and Spinal Ganglia and the Viscero-Motor Roots in Amphioxus. Biol. Bull., Vol. 9, No. 2, pp. 112–127.

Kowalewsky, A.

'67. Entwickelungsgeschichte des Amphioxus lanceolatus. Mém. Acad. impér. Sci. St. Pétersbourg, Sér. 7, Tom. 11, No. 4, 17 pp. 3 Taf.

Kupffer, K. von.

: 03–05. Die Morphogenie des Centralnervensystems. Handbuch vergl. u. exper. Entwickelungslehre Wirbeltiere, Bd. 2, Theil 3, pp. 1–272. (Amphioxus pp. 1–12, 1903.)

Langerhans, P.

'76. Zur Anatomie des Amphioxus lanceolatus. Arch. f. mikr. Anat., Bd. 12, pp. 290–348, Taf. 12–15.

Leuckart, R., und Pagenstecher, A.

'58. Untersuchungen über niedere Seethiere. Arch. f. Anat., Physiol. u. wiss. Med., Jahrg. 1858, pp. 558–613, Taf. 18–23. (Amphioxus, pp. 558–569, Taf. 18.)

Lönnberg, E.

: 01—. Pisces. Bronn's Klassen u. Ordnungen des Thier-Reichs, Bd. 6, Abth. 1. (Leptocardii Lief. 2–20, pp. 99–249, Taf. 1–12, 1902–1905.)

Macbride, E. W.

: 00. Further Remarks on the Development of Amphioxus. Quart. Jour. Micr. Sci., Vol. 43, (N. S.). pp. 351–366, pl. 17.

Mallory, F. B.

: 00. A Contribution to Staining Methods. Jour. Exp. Med., Vol. 5, No. 1, pp. 15–20. *Also abstr.* in Jour. Appl. Micr., Vol. 3, pp. 1036–1038.

Marcusen, T.

'64. Sur l'anatomie e l'histologie du Branchiostoma lumbricum. Costa (Amphioxus lanceolatus. Yarrell). Comptes Rendus Acad. Sci., Paris, Tom. 58, pp. 479–483. *Also transl. in* Ann. and Mag. Nat. Hist., Ser. 3, Vol. 14, pp. 151–154, 1864.

Müller, J.

'41. Mikroskopische Untersuchungen ueber den Bau und die Lebenserscheinungen des Branchiostoma lumbricum Costa, Amphioxus lanceolatus Yarrell. Monatsbericht über die zur Bekanntmachung geeigneten Verhandlungen. Königl. Preuss. Akad. Wissensch. zu Berlin, 1841, pp. 396–411. *Also sep.* 16 pp. [Berlin] 1841.

Müller, J.

'44. Ueber den Bau und die Lebenserscheinungen des Branchiostoma lumbricum Costa, Amphioxus lanceolatus Yarrell. Abhandl. Königl. Akad. Wissensch. zu Berlin. a. d. Jahre 1842, Physikal. Abhandl., pp. 79–116, 5 Taf. 1844.

Nüsslin, O.

'77. Zur Kritik des Amphioxusauges. Inaug.-Diss., Tübingen, 33 pp., 2 Taf.

Owsjannikow, P.

'68. Ueber das Centralnervensystem des Amphioxus lanceolatus. Bull. Acad. Impér. Sci. St. Pétersbourg, Tom. 12, pp. 287–302, 1 Taf.

Platt, Julia.

'92. Fibres connecting the Central Nervous System and Chorda in Amphioxus. Anat. Anz., Jahrg. 7, No. 9 u. 10, pp. 282–284.

Quatrefages, A. de.

'45. Mémoire sur le Système nerveux et sur l'Histologie du Branchiostome ou Amphioxus. Ann. Sci. Nat., Zool., Sér. 3, Tom. 4, pp. 197–248, pl. 10–13.

Rathke, H.

'41. Bemerkungen über den Bau des Amphioxus lanceolatus, eines Fisches aus der Ordnung der Cyclostomen. Königsberg, 38 pp., 1 Taf.

Reichert, C. B.

'70. Zur Anatomie des Branchiostoma lumbricum. Arch. f. Anat., Physiol. u. wiss. Med., Jahrg. 1870, pp. 755–758.

Retzius, G.

'91. Zur Kenntniss des centralen Nervensystems von Amphioxus lanceolatus. Biolog. Untersuch., N. F., Bd. 2, No. 2, pp. 29–46, Taf. 11–14.

Retzius, G.

'98. Die Methyleneblaufärbung bei dem lebenden Amphioxus. Biol. Untersuch., N. F., Bd. 8, No. 14, pp. 118–122.

Rohde, E.

'88. Histologische Untersuchungen über das Nervensystem von Amphioxus lanceolatus. Zool. Beiträge (Schneider), Bd. 2, Heft 2, pp. 169–211, Taf. 15, 16.

Rohon, J. V.

'82. Untersuchungen über Amphioxus lanceolatus. Denkschr. Akad. Wiss. Wien, Math.-Naturw. Cl., Bd. 45, 64 pp., 6 Taf.

Rolph, W.

'76. Untersuchungen über den Bau des Amphioxus lanceolatus. Morph. Jahrb., Bd. 2, pp. 87–164, Taf. 5–7.

Schneider, A.

'79. Beiträge zur vergleichenden Anatomie und Entwicklungsgeschichte der Wirbelthiere. Berlin, viii+164 pp., 16 Taf. (Amphioxus, pp. 1–31, Taf. 14–16.)

Schneider, A.

'80. Ueber die Nerven von Amphioxus, Ammocoetes und Petromyzon. Zool. Anz., Jahrg. 3, pp. 330–334.

Stieda, L.

'73. Studien über den Amphioxus lanceolatus. Mém. Acad. Impér. Sci. St. Pétersbourg, Sér. 7, Tom. 19, No. 7, 71 pp., 4 Taf.

Wijhe, J. W. van.

'84. Ueber den vorderen Neuroporus und die Phylogenetische Function des Canalis neurentericus der Wirbelthiere. Zool. Anz., Jahrg. 7, pp. 683–687.

Wijhe, J. W. van.

'93. Ueber Amphioxus. Anat. Anz., Jahrg. 8, pp. 152–172.

Willey, A.

'94. Amphioxus and the Ancestry of the Vertebrates. Macmillan and Co., New York and London, xiv+316 pp., and frontispiece.

EXPLANATION OF FIGURES.

Each drawing was outlined with an Abbé camera lucida. The numbers of the dorsal nerves are indicated by Roman numerals. Unless otherwise stated, dorsal is up in all figures.

Abbreviations.

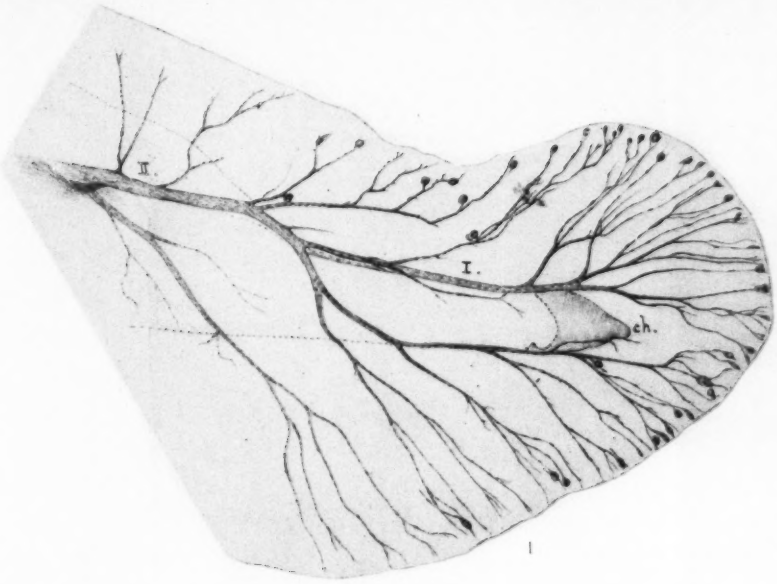
N. B.— Unfortunately an entirely consistent system of lettering figures could not be adopted owing to the incorporation of the provisional lettering in the drawings themselves. With the reduction in size accompanying the reproduction of the figures, many of these letters are extremely small. For explanation of all letters not embraced in the following list, the reader may consult the explanation of the Figure on which the letters occur.

ant.	Anterior.	*n.*	Nucleus.
b. n.	Band nerve of velum.	*n. t.*	Neural tube.
brs. gon.	Gonadial pouch.	*o.*	Nerve branches to outer mouth plexus. In Fig. 2, *o* is olfactory pit.
ch.	Chorda dorsalis.		
dx.	Right.		
e. p.	End-plate.	*par. go.*	Wall of gonadium.
e. s.	Eye-spot.	*post.*	Posterior.
i.	Nerve branches to inner mouth plexus.	*Q.*	Cells of Quatrefages.
		r. c.	Ramus cutaneus.
i. a. p.	Inner abdominal plexus.	*r. c. v.*	Ramus cutaneus ventralis.
l.	Lateral.		
l. d.	Ligamentum denticulatum.	*r. d.*	Ramus dorsalis.
		r. v.	Ramus ventralis.
m.	Muscle.	*r. v. a.*	Ramus visceralis ascendens.
m_1, m_2, m_3.	Muscle of myomeres 1, 2, 3 etc.	*r. v. d.*	Ramus visceralis descendens.
marg. l.	Lateral margin.		
marg. m.	Median margin.	*sin.*	Left.
med.	Median.	$t.^1$	Velar tentacle, small.
m. v.	Ventral margin of muscle.	$t.^2$	Velar tentacle, large, central.
my'sep.	Myoseptum.	*v.*	Velum.

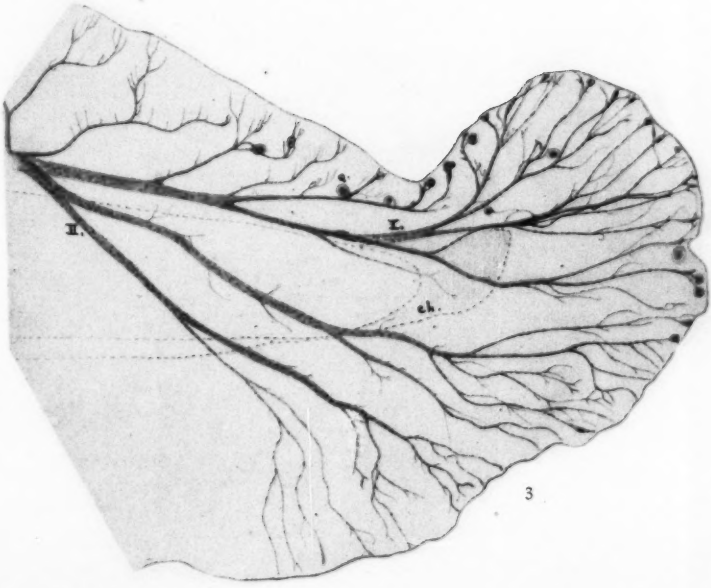
PLATE 1.

All figures are from methylene-blue preparations of *Branchiostoma caribaeum;* the epithelium of the skin is not present.

FIGURE 1. Nerves of the right side of the rostrum. × 93.
FIGURE 2. Nerves of the left side of the rostrum. × 93.
FIGURE 3. Nerves of the right side of the rostrum. A few nerve branches in the ventral region are not shown. × 93.
FIGURE 4. Nerves of the left side of the rostrum. × 93.

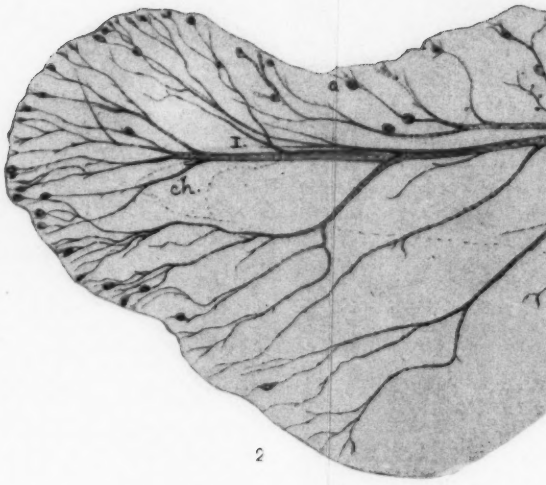


1

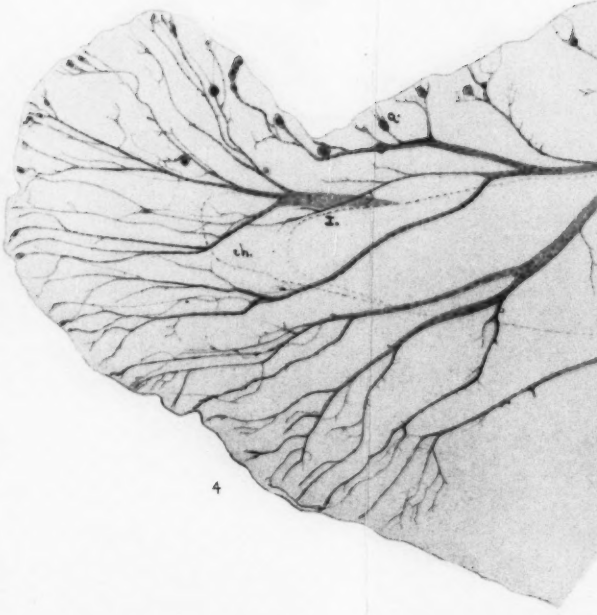


3

H. L. del.

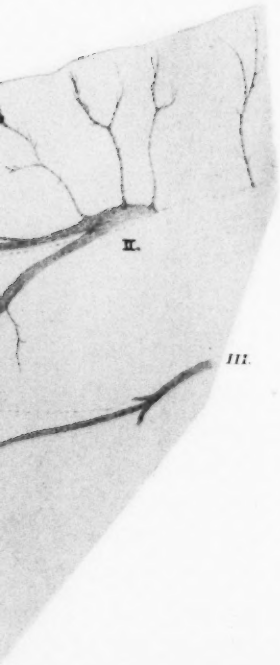


2



4

PLATE I



HELIOTYPE CO., BOSTON.

PLATE 2.

All figures are from methylene-blue preparations.

FIGURE 5. Branchiostoma caribaeum. Nerves of the right side of the rostrum. The epithelium of the skin is not present. Numerous cells of Quatrefages, *Q*, *Q*, are shown. × 92.

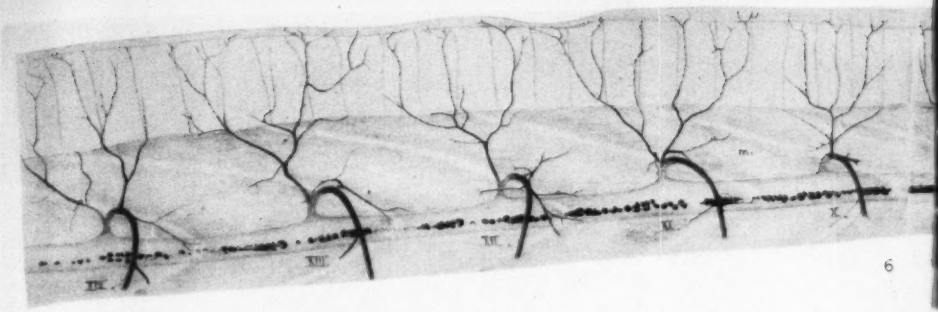
FIGURE 6. B. caribaeum. Anterior dorsal portion of a transparent specimen, showing the exit of the anterior dorsal nerves from the neural tube on the right side of the body. The branches of the dorsal rami of these nerves are drawn. Nerve II shows two roots. The places of division of these nerves into dorsal and ventral rami are also shown. × 32.

FIGURE 7. B. caribaeum. The main branches of the dorsal nerves of the buccal region, on the left side of the body. The branches *r*, *r*, pass to the right side of the body; *a*, *a*, indicate places of anastomosis. The branches *o*, *o*, form part of the outer mouth plexus; those marked *i*, *i*, form part of the inner mouth plexus; *v*. band nerve of velum. × 34.

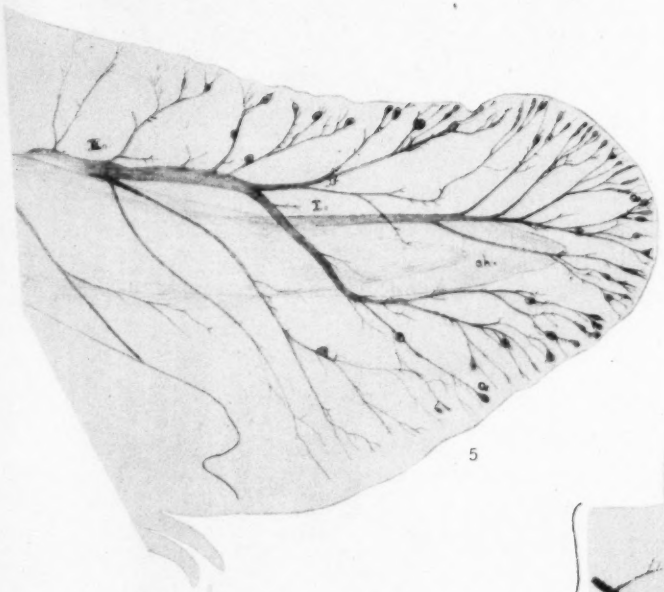
FIGURE 8. B. lanceolatum. Anterior is up. The innervation of the velum. The epithelium of the velum is not present. The velar tentacles are indicated by t^1, t^2; *a*, *a*, point where one velar nerve band was cut; *b*, *b*, similar point in another cut nerve band; *b'*, the cut nerve *b* near the point where it diverges from δ. For nerves α, β, γ, δ see text p. 590. × 41.

FIGURE 9. B. lanceolatum. Anterior is up. A portion of the velar plexus, and the nerves of two velar tentacles; *c*, *c*, cells in the epithelial covering remaining attached to the nerves. Cells, or nuclei, in connection with the velar plexus are shown at *n*, *n*. × 123.

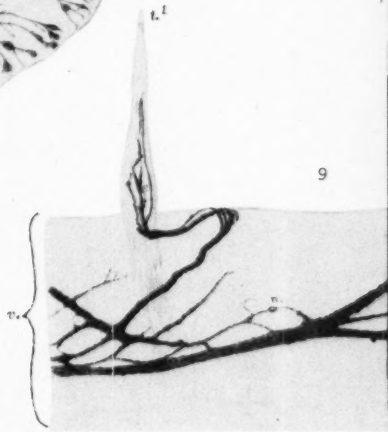
FIGURE 10. B. lanceolatum. A portion of the fine plexus of a gonadial pouch. × 343.



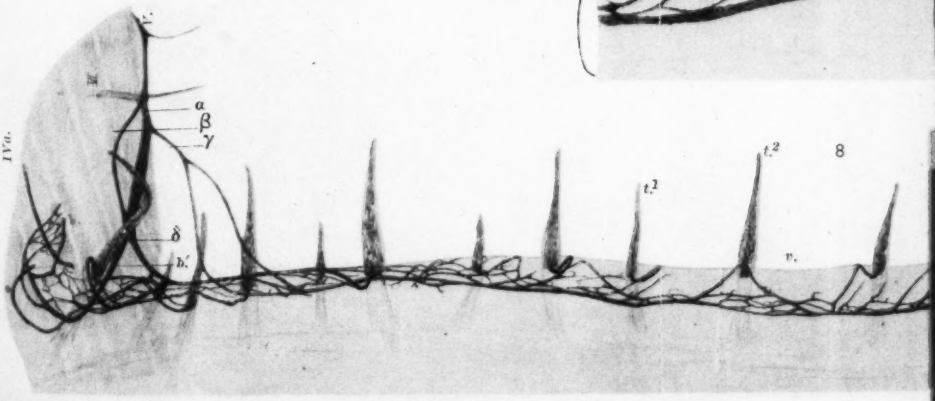
6



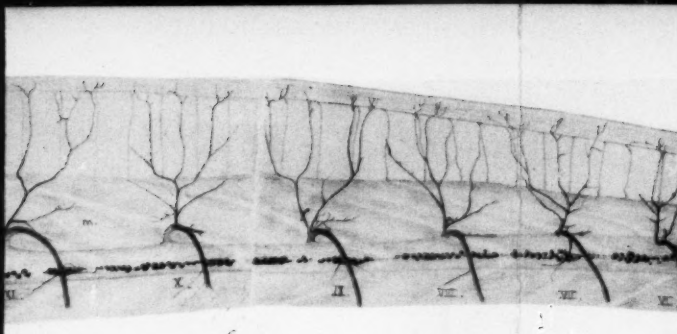
5



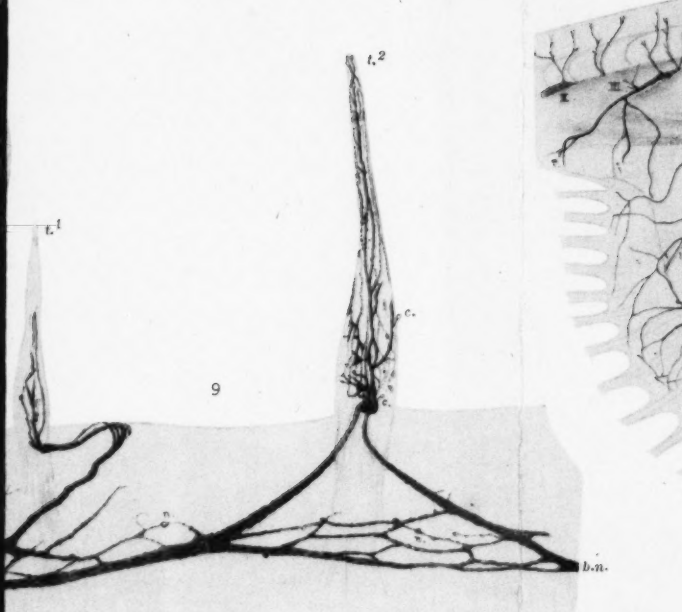
9

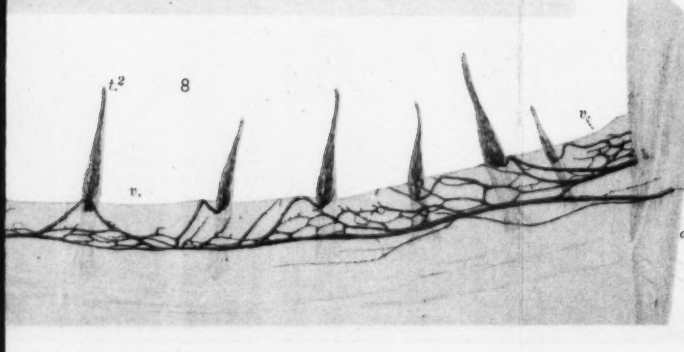


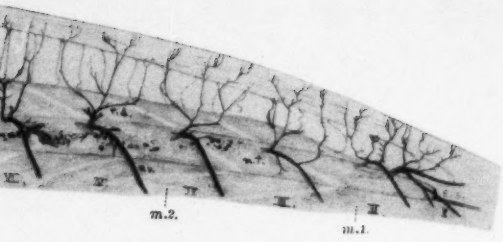
8

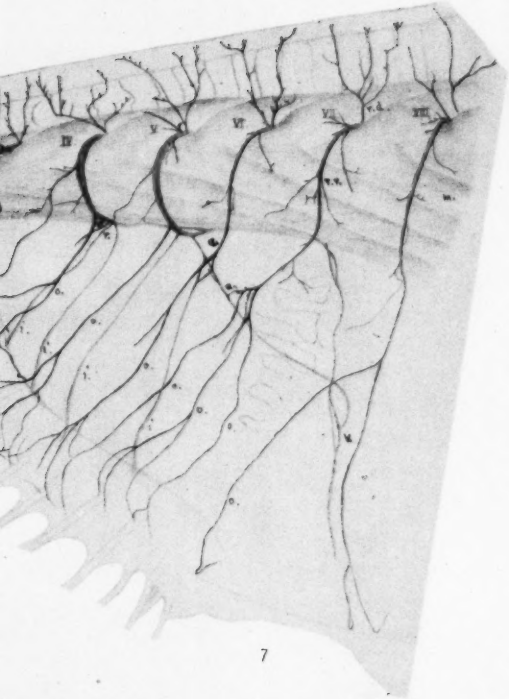


6



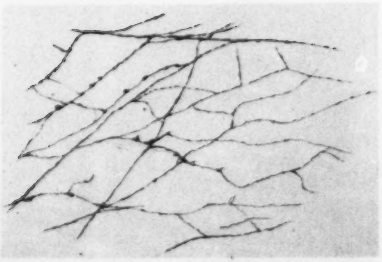






7

10



HELIOTYPE CO., BOSTON.

PLATE 3.

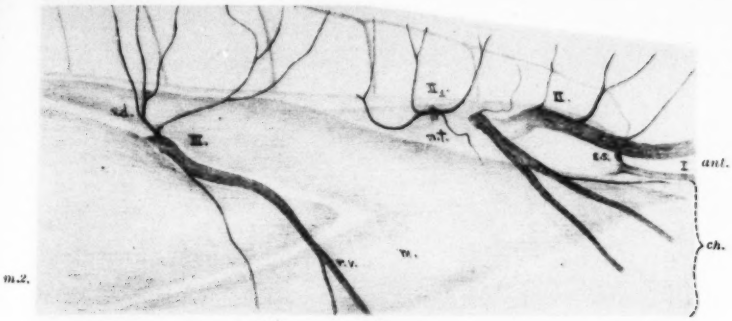
All figures are from methylene-blue preparations.

FIGURE 11. Branchiostoma lanceolatum. The first and second (second and third of Hatschek) myomeres, m_1, and m_2, and the proximal portions of nerves I, II and III, of the *right* side. Nerve II shows two roots and perhaps a third (between II and II_1). × 84.

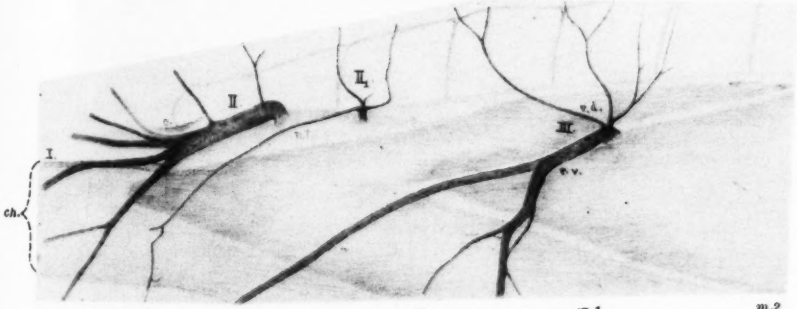
FIGURE 12. B. lanceolatum. The first and second myomeres, m_1 and m_2, and the proximal portions of nerves I, II and III, of the *left* side. Nerve II shows two roots (II and II_1); *o*. olfactory pit. × 84.

FIGURE 13. B. lanceolatum. The visceral rami of a dorsal nerve, viewed from the interior. The branches supplying a gonadial pouch (*brs. gon.*) are shown. The larger meshes of the plexus on the interior (median) surface of this pouch are drawn. The place of emergence of the visceral ramus on the internal surface of the side muscles is indicated at *a*. The branches *i*, *i*, from ramus visceralis descendens pass to the gonadial pouch. The ventral muscle border is indicated at *m. v.* × 123.

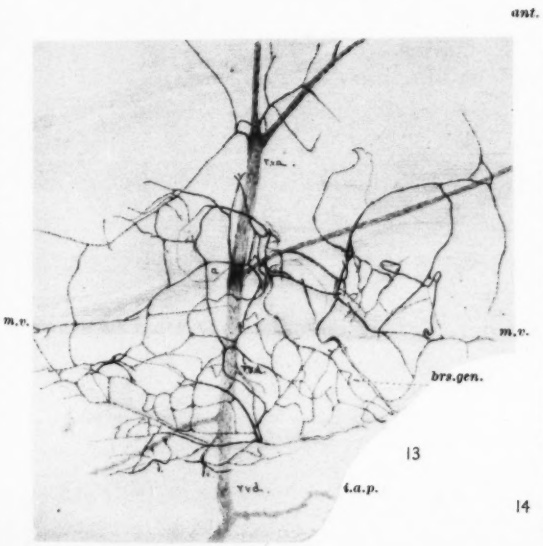
FIGURE 14. B. lanceolatum. A nerve plexus on a secondary branchial bar, not far from the ligamentum denticulatum, viewed from the external (lateral) surface of the branchial basket. Branchial nerves of the anterior part of the animal. × 254.



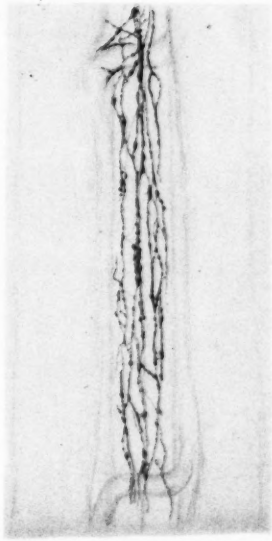
11



12



13



14

H. L. del

HELIOTYPE CO. BOSTON.

PLATE 4.

All figures, except 15a, from methylene-blue preparations.

FIGURE 15. Branchiostoma lanceolatum. The fan-like branches of an ascending visceral ramus of a dorsal nerve, viewed from the interior. The visceral ramus reaches the internal surface of the side muscles at *a*. The branches at *c* and *d* anastomose with those of adjoining ascending visceral rami. The branch at *e* bends toward the exterior. The branches at *b* are bent away from the muscle, i. e. toward the median plane of the animal. × 93.

FIGURE 15a. Diagram of part of a cross section of an Amphioxus, to show the course of a dorsal nerve and its branches.

FIGURE 16. B. lanceolatum. View of the inner (median, or atrial) face of the lateral muscles of right side (lower half of figure), and of outer (atrial) surface of the branchial wall (*p. w.*), cut ventrally and turned up (the upper half of figure). To show branches of an ascending visceral ramus passing to the pharynx. Portions of the ligamentum denticulatum are marked *l. d.* The plexus formed by the fan-like branches of an ascending visceral ramus is also shown. × 84.

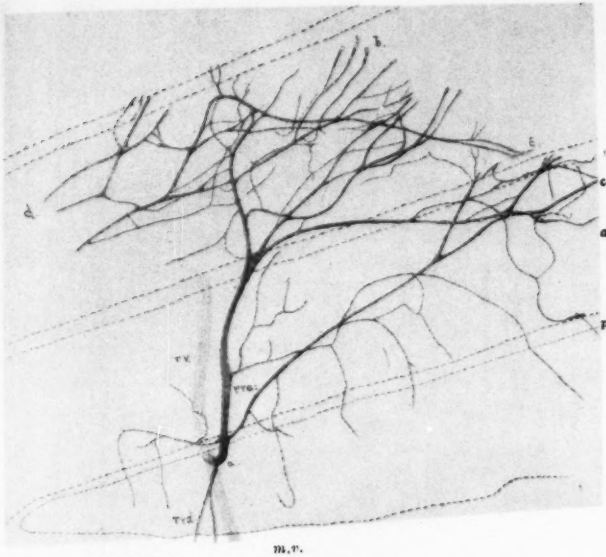
FIGURE 17. B. lanceolatum. Similar view to that of Figure 16. Nerves entering the pharynx from the ligamentum denticulatum, and a portion of the plexus of the latter structure. The plexus in the "pocket" portions of the ligament is shown at *pl. d.* The plexus of a primary branchial bar is marked *b. p. 1*, and that of a secondary bar, *b. p. 2.* × 177.

FIGURE 18. Branchiostoma caribaeum. Anterior is up. A part of the outer plexus of the mouth border, on the *right* side of the body. × 93.

FIGURE 19. B. lanceolatum. Plexuses on a primary bar, a secondary bar, and a cross-bar of the pharynx, with nerve cells, *c*, *c*, in connection. This is a view of the exterior surface of a part of the pharynx which is near its ventral surface and near the anterior end of the body on the right side; *a*, and *b*, have the same meaning as in the following figure. × 254.

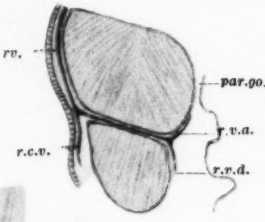
FIGURE 20. B. lanceolatum. A view of the dorsal border of a portion of the left side of the pharynx from the interior of the latter, showing a nerve plexus. The primary bars are marked *a*, *a*, and the secondary bar, *b*. × 254.

FIGURE 21. B. lanceolatum. A nerve cell on the external side of a secondary bar of the pharynx. The view is from the right side of the latter, near the anterior end of the body. × 700.

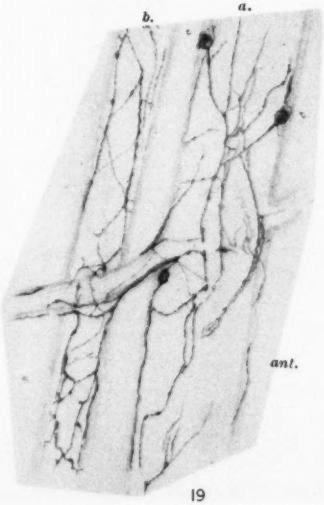


15

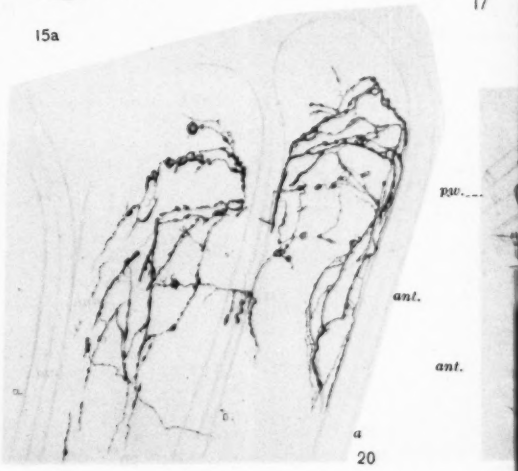
17



15a

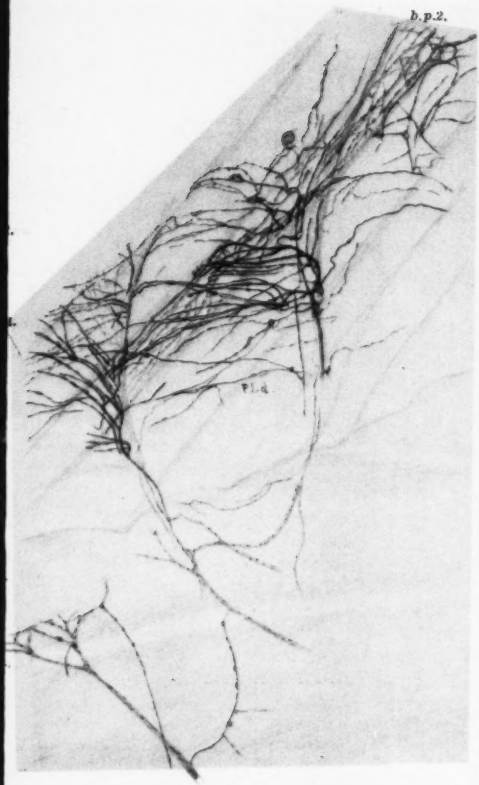


19



20

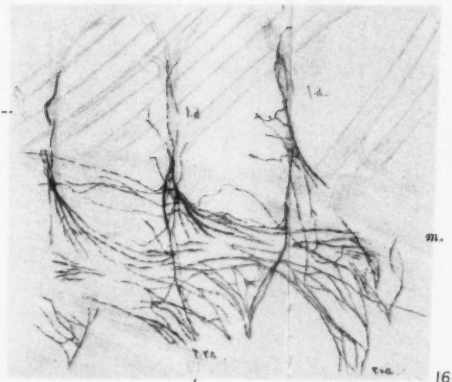
H. L. del.



17

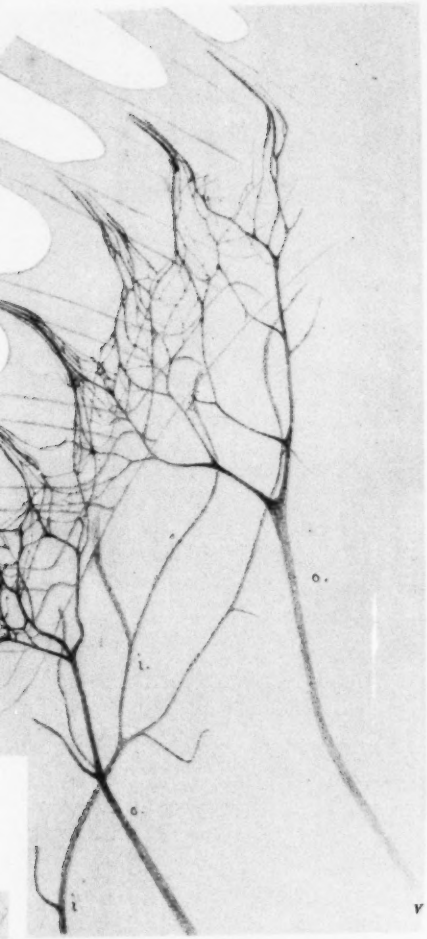
18

20

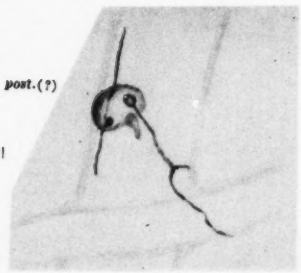


16

PLATE 4



IV sin. *IV dx.*



21

16

HELIOTYPE CO., BOSTON

PLATE 5.

Figure 24 from gold-choride preparation; all others from Golgi preparations.

FIGURE 22. Branchiostoma caribaeum. Transverse section. Nerve fibers entering a dorsal root, in the middle region of the animal. × 238.

FIGURE 23. B. caribaeum. Anterior at the *left* (?). A frontal section. Nerves in the transverse muscles, toward the anterior end of the body, on the right side. × 238.

FIGURE 24. B. caribaeum. A view of the inner surface of skin stripped from the dorsal fin in the region of the 20th myomere from the posterior end of the animal, showing fibers and other structures in connection with a branch of the dorsal ramus of a dorsal nerve. Ordinary epithelial cells are outlined at *e*. Special cells in the epithelial layer are marked *g*, and certain cell-like structures in connection with fibers are shown at *c*, *c*. A star-like place of union of fibers is indicated at *s*. The bases of the epithelial cells lie just exterior to the fibers drawn. × 410.

FIGURE 25. B. lanceolatum. Dorsal aspect of frontal section through the 7th myomere of the right side showing a portion of the corresponding ventral nerve root. × 203.

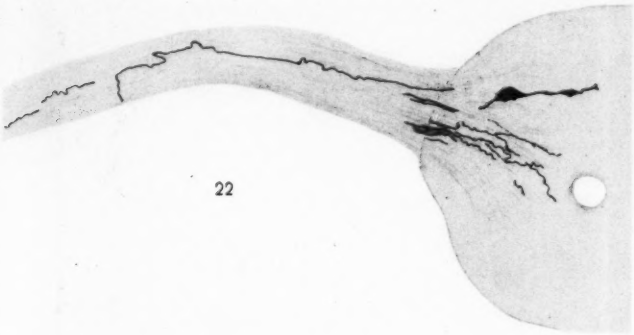
FIGURE 26. B. lanceolatum. Dorsal aspect of frontal section, next ventral to the preceding one, showing a more ventral portion of the same nerve. × 203.

FIGURE 27. B. lanceolatum. Frontal section at the level of the dorsal margin of the notochord, directly ventral to the neural tube. The myomere of the figure is slightly posterior to the middle of the specimen, on the right side. Smooth motor fibers are shown, and a closely associated group of end-plates situated in the side muscles, near the posterior myoseptum. × 141.

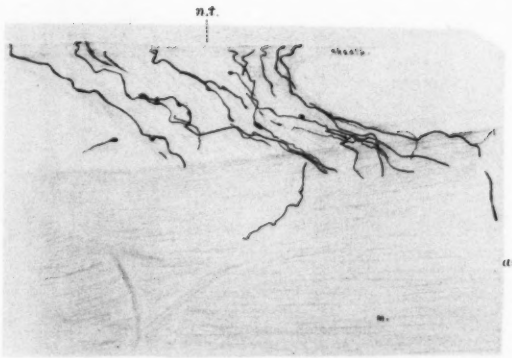
FIGURE 28. B. lanceolatum. Frontal section through the middle of the notochord, showing long motor fibers and end-plates in the side muscles. These muscles are posterior to the middle region of the body. × 141.

FIGURE 29. B. lanceolatum. Dorsal aspect of frontal section, showing motor fibers in the side muscles of the 18th myomere of the right side, in the region of the neural tube just ventral to the place of exit of the dorsal nerve roots. The section is about 46 micra in thickness. × 99.

FIGURE 30. Branchiostoma caribaeum. A nerve cell in the ventral part of the pharynx, in connection with the nerve plexus of that region. The cell lies on the exterior of an endostylar plate. × 226.

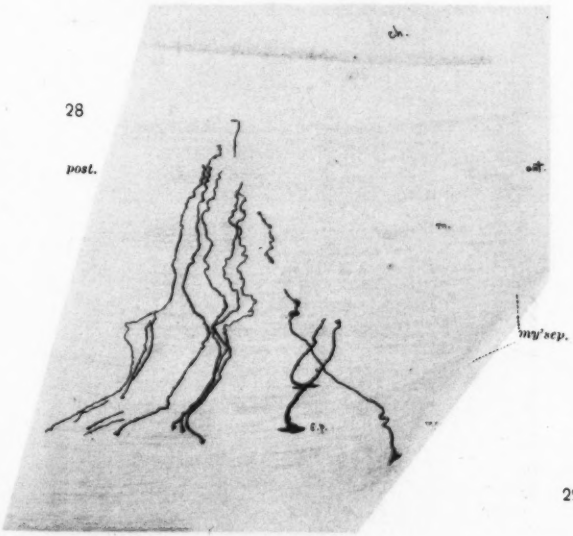


22



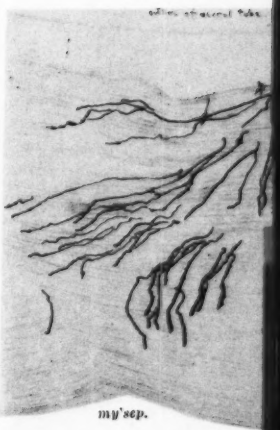
25

26

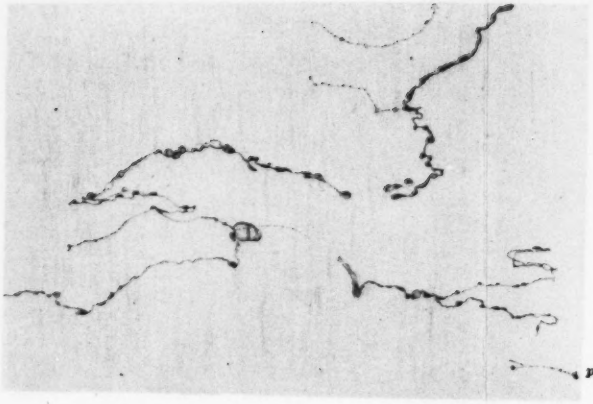


28

29

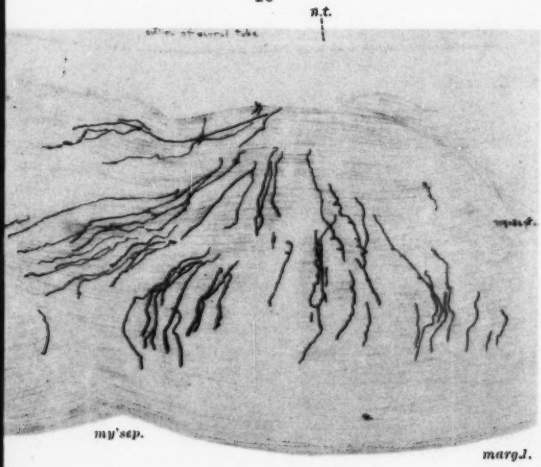


H L del.



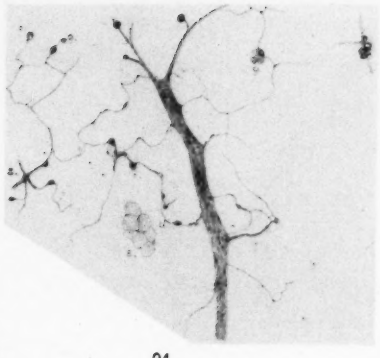
23

26



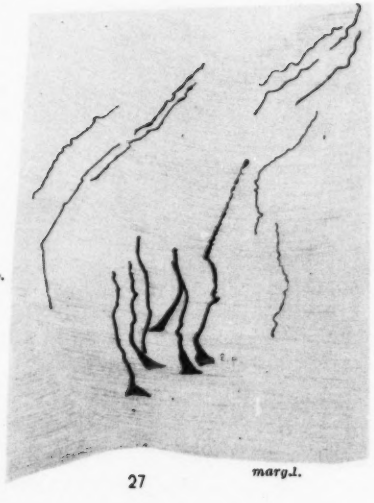
30

PLATE 5



post.(?)

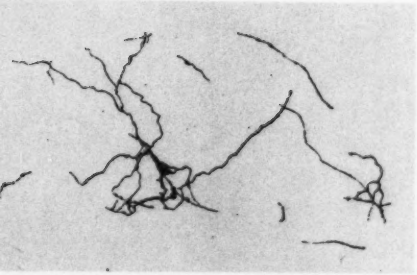
24



y'sep.

marg.l.

27



HELIOTYPE CO., BOSTON.

PLATE 6.

All figures are from methylene-blue preparations.

FIGURE 31. Branchiostoma caribaeum. The same individual as that shown in Figure 33. The nerves of the posterior end, on the right side of the body. × 93.

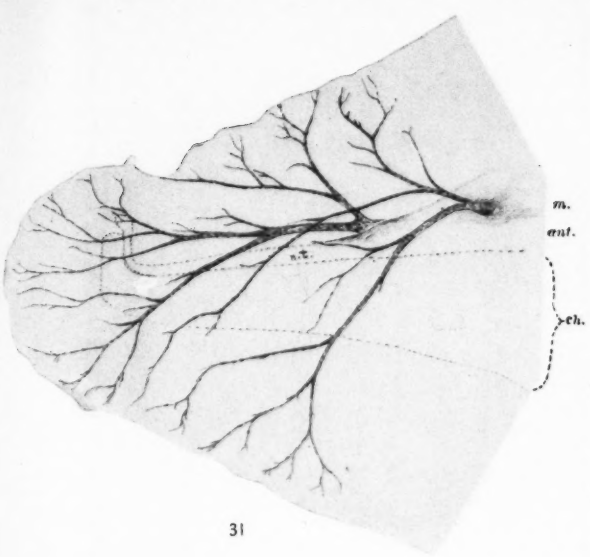
FIGURE 32. B. lanceolatum. The interior surface of the ventral fin and side muscles bordering upon it, from the region between the atriopore and the anus. Posterior is up. The branches from the ventral rami of dorsal nerves which reach the interior are marked *a*, *a*, *a*. The branch a_1, bends toward the exterior. Part of a membrane, somewhat detached from the side muscles, is indicated at *b*. The bilateral asymmetry of the muscle segments is illustrated. × 84.

FIGURE 33. B. caribaeum. Nerves of the posterior end of the body, on the left side. The epithelium of the skin is not present. The end of the notochord is bent in the same manner as the terminal ampulla of the neural tube. × 93.

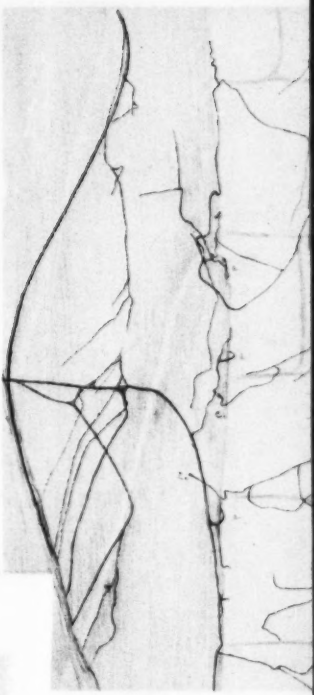
FIGURE 34. B. lanceolatum. Cutaneous plexuses between right dorsal nerves XLVI, XLVII and XLVIII, in the region of the side muscles. The coarser, deeper plexus is marked p_1 (center of figure), the finer, more superficial one p_2 (upper left quarter of figure). A cell-like structure is shown at *c*, in connection with cutaneous nerve fibers and at *a*, a nerve passing through myoseptum to inner face of muscle. × 41.

FIGURE 35. B. lanceolatum. Cutaneous plexuses between right dorsal nerves LIII and LIV, and between LV and LVI, in the region of the side muscles. The letters correspond to those of Figure 34. The branch at *x* joins nerve LVII. × 41.

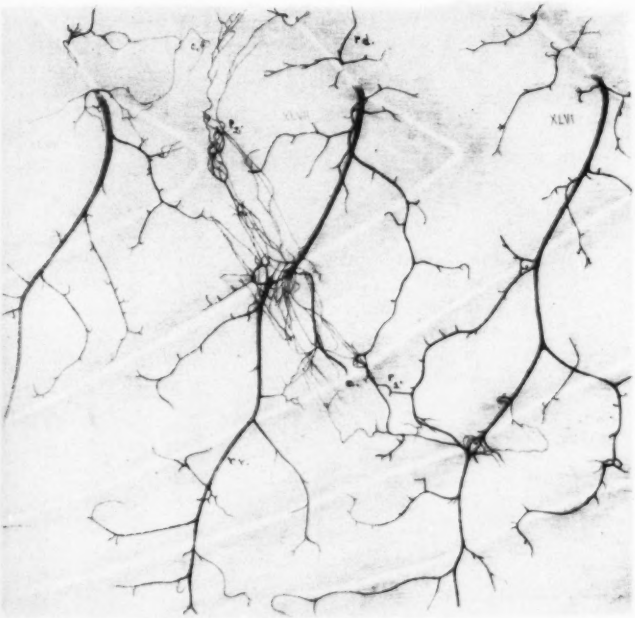
FIGURE 36. B. lanceolatum. Cutaneous plexuses between right dorsal nerves LIV and LV, in the region of the side muscles. The letters correspond to those of Figure 34. × 84.



31

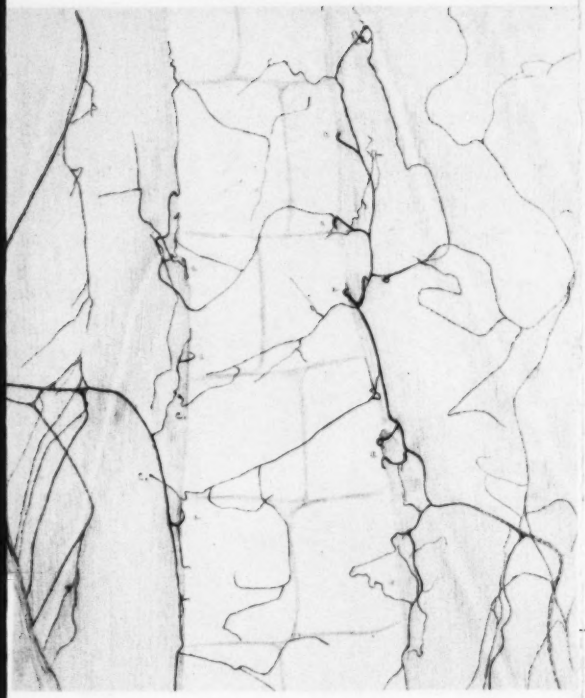


34



H. L. del.

ventral fin.



32

post.

35

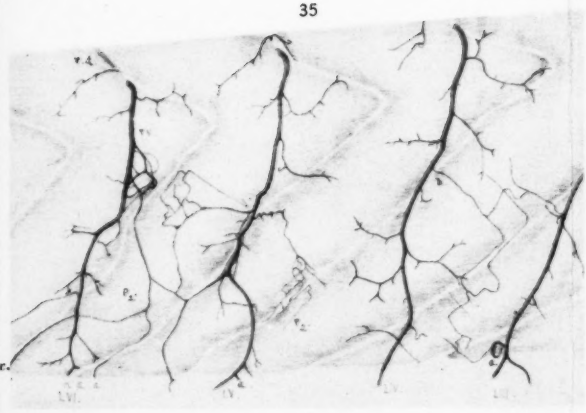
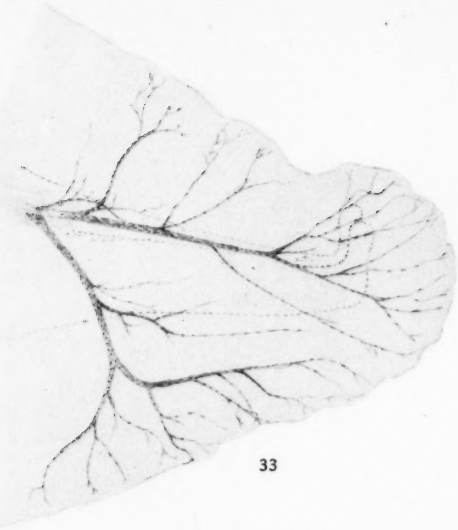


PLATE 6



33

36

HELIOTYPE CO., BOSTON.

PLATE 7.

Figures 37–39 and 41 from methylene-blue preparations; figure 40 from Golgi, and figure 42 from gold-chloride (after Ranvier) preparations.

FIGURE 37. Branchiostoma lanceolatum. The exteriorly directed cutaneous branchlets of left dorsal nerve XVI. The epithelium is not present. × 41.

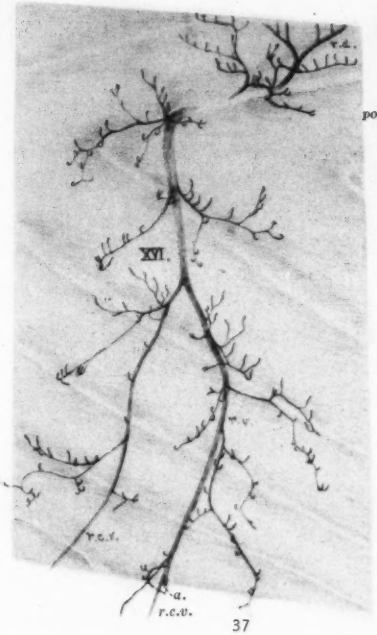
FIGURE 38. B. lanceolatum. Cutaneous plexuses distributed over the side muscles, formed from connecting branches of left nerves XI, and XII. The coarser plexus is marked p_1, and the finer, more superficial plexus, p_2. A visceral nerve branch is indicated at *a*. × 84.

FIGURE 39. B. caribaeum. Cutaneous plexuses between left dorsal nerves XXXV, XXXVI and XXXVII, in the region of the side muscles. The letters correspond to those in preceding figure. × 17.5.

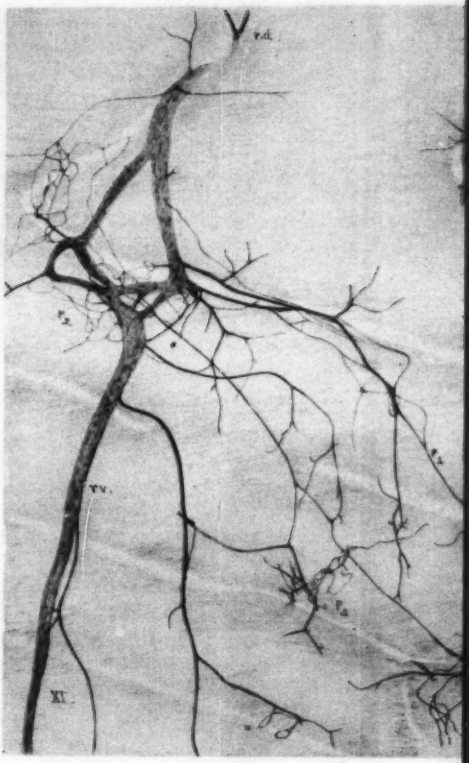
FIGURE 40. B. lanceolatum. Anterior is up. Frontal section. Nerve fibers entering the root of right dorsal nerve XXI. × 238.

FIGURE 41. B. lanceolatum. Dorsal is at the *left*. A plexus formed by the breaking up, for a short distance, of the main stem of the ventral ramus of right dorsal nerve XXVII. × 84.

FIGURE 42. B. caribaeum. A view of the outer surface of skin taken from the region over the side muscles, near the dorsal fin. Special cells are indicated at *g*, and modified cells at *s*. Apparent exudations from the special cells are marked *e*. The exudations lie at higher focus in their distal portions. × 1050.

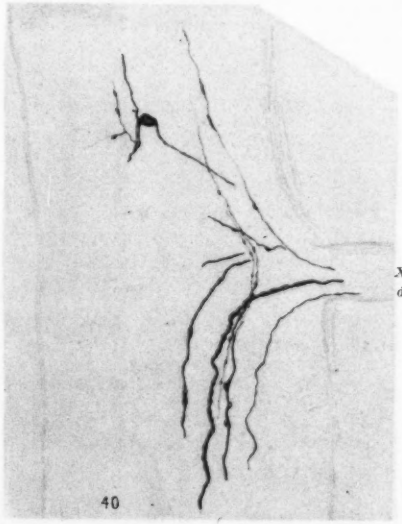


37

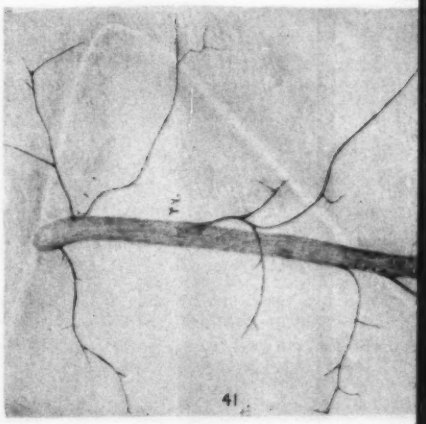


38

ant.

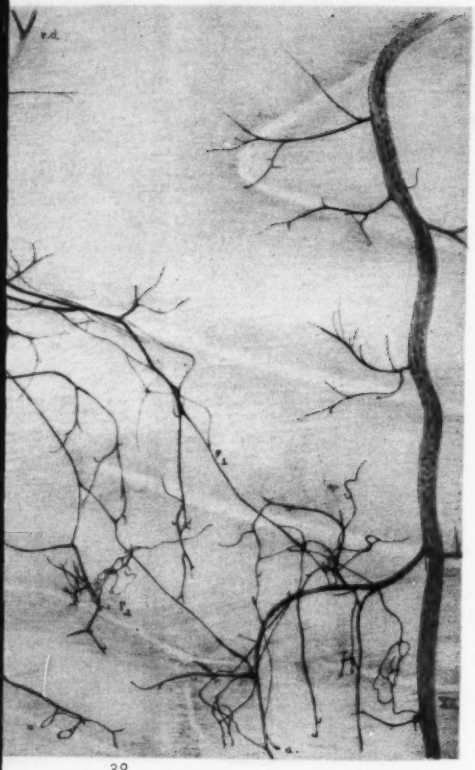


40

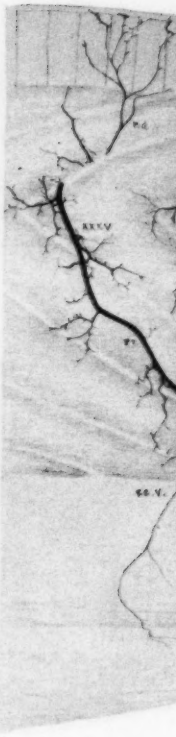


41

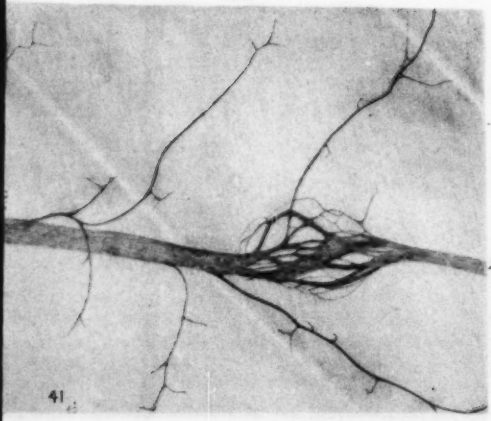
H. L. del.



38



ant.



41

ventral.

XXVII.

c.

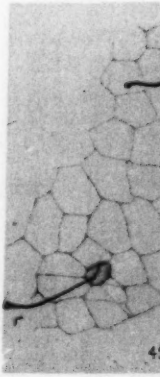
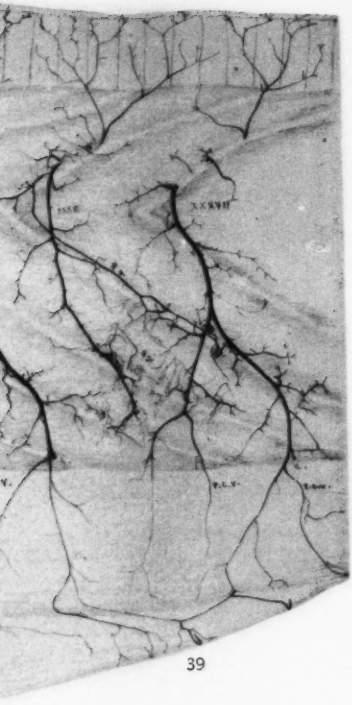
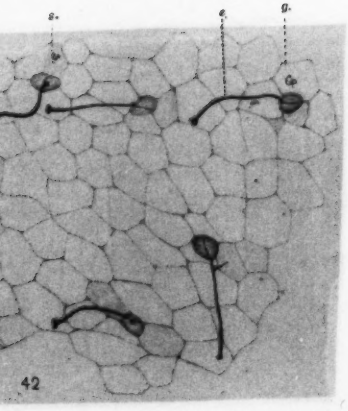


PLATE 7



39



42

HELIOTYPE CO., BOSTON.

PLATE 8.

All figures are from Golgi preparations, and all except 50 are from Branchiostoma lanceolatum and are dorsal aspects of frontal sections.

FIGURE 43. Section (anterior at the right) through the middle of the notochord (*ch.* sheath of notochord), showing part of a myomere in the middle region of the animal. Long, single motor fibers are drawn, the one bearing an end-plate traversing the side muscles for nearly their entire width. × 141.

FIGURE 44. Section (anterior at the left) through the 24th myomere of the left side, showing motor fibers and an end-plate. A slight impregnation of the muscle fibers adjoining the end-plate is drawn. × 203.

FIGURE 45. Section showing motor end-plates in the side muscles dorsal to the neural tube, on the left side. × 277.

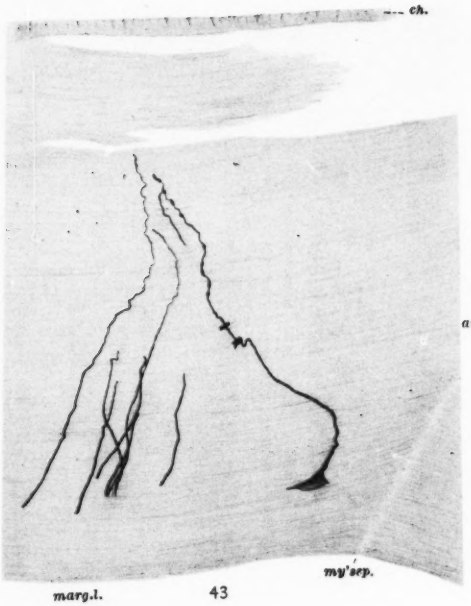
FIGURE 46. Section (anterior at the left) showing long, smooth, closely associated motor fibers in the middle region of the 20th myomere of the left side of the body. The section passes through the notochord. × 141.

FIGURE 47. Section (anterior at the right) through the region of the notochord, showing long, smooth motor fibers near a myoseptum. × 141.

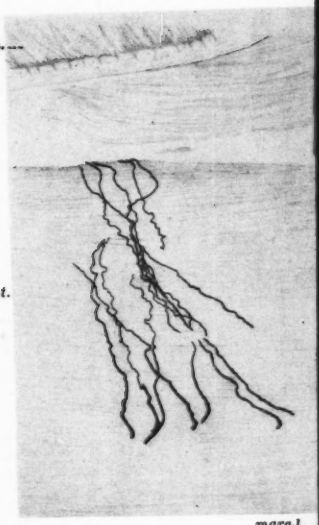
FIGURE 48. Section (anterior at the left) through the region of the 21st myomere of the left side of the body, showing a branched motor fiber near the anterior myoseptum. × 203.

FIGURE 49. Frontal section through the region of the 19th myomere of the left side, showing branched motor fibers near a myoseptum. × 203.

FIGURE 50. B. caribaeum. Transverse section through a ventral nerve near the middle region of the body, showing a nerve fiber bearing a structure (*c*) resembling a bipolar nerve cell. An apparent nucleus is shown in the cell-like structure. × 271.

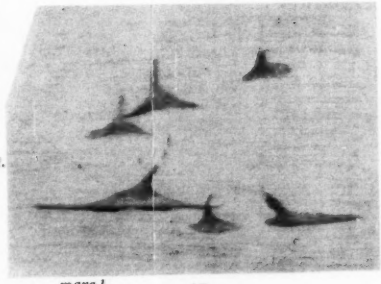


43



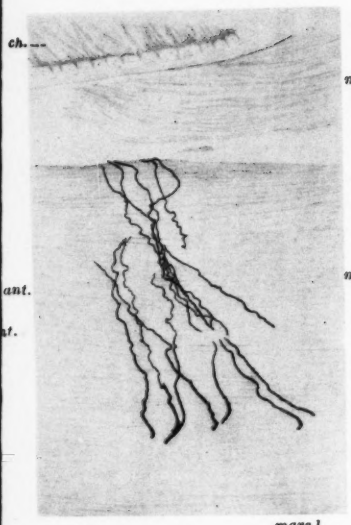
46

44

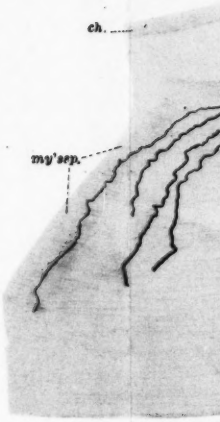


45

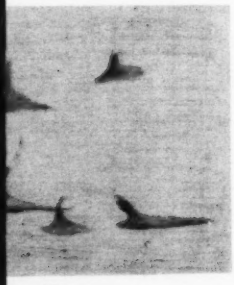
H. L. del.



46



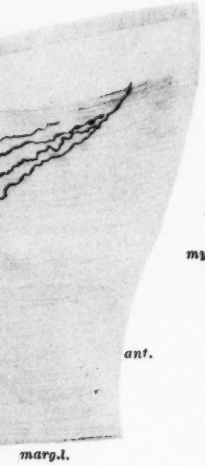
47

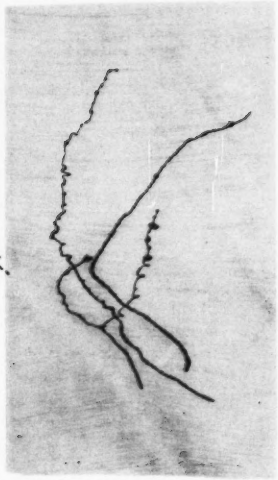


45

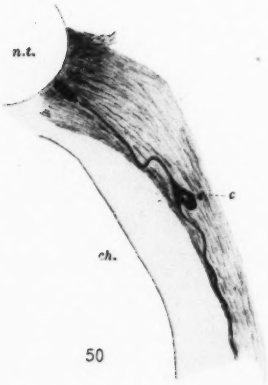
49

PLATE 8





48



50

HELIOTYPE CO., BOSTON.